CALIFORNIA FISH AND GAME

"CONSERVATION THROUGH EDUCATION"

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PROLIFERATIVE KIDNEY DISEASE (TETRACAPSULOIDES BRYOSALMONAE) IN MERCED RIVER HATCHERY JUVENILE CHINOOK SALMON: MORTALITY AND PERFORMANCE IMPAIRMENT IN 2005 SMOLTS

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ABSTRACT

Merced River Hatchery (MRH) juvenile Chinook salmon, from tagged lots used in the 2005 Vernalis Adaptive Management Program (VAMP) study, were brought to the California-Nevada Fish Health Center wet lab 6 days prior to the first VAMP release and reared for 50 days at water temperatures similar to the San Joaquin River. At the time of transport, a fish health inspection showed that the population was generally healthy but had a low prevalence of an early stage infection by the myxosporean parasite, *Tetracapsuloides bryosalmonae*. This parasite has been detected in Merced River salmon for several decades and causes Proliferative Kidney Disease (PKD). The level of clinical PKD, as demonstrated by a combined kidney lesion and anemia score, markedly increased starting at 29 days post-transfer (dpt) from MRH. Severe disease occurred in the study population after the last VAMP coded wire tag fish was recovered in the Chipp's Island trawl on 27 May. A total of 76 study salmon died (27% cumulative mortality) due to PKD beginning at 36 dpt through the final sample at 50 dpt. Both dpt and disease state correlated with a decline in hematocrit and plasma magnesium as well as an elevation in circulating white blood cell number and plasma protein concentration. There was no observed PKD effect on time to exhaustion during a 120-min swim challenge until 50 dpt. Smolt development measurements indicated that the study fish were in an advanced stage of smoltification. Similar to swim performance, saltwater adaptation was not impaired until 50 dpt. *Tetracapsuloides bryosalmonae* was observed in 40% (17 of 43) of the kidney imprints collected from VAMP tag salmon recovered in the Chipp's Island trawl. These results indicate that while *T. bryosalmonae* infection is prevalent in Merced River out-migrant salmon, it may not have a significant effect on VAMP recoveries. However, PKD could be a significant mortality factor for Merced River salmon smolts during their early seaward entry phase.

INTRODUCTION

Proliferative Kidney Disease (PKD) has been diagnosed in Merced River Hatchery (MRH) juvenile Chinook salmon, *Oncorhynchus tshawytscha*, for several decades (Hedrick et al. 1986). The incidence of *T. bryosalmonae* infection in MRH salmon inspected prior to and shortly after release has ranged from 4 – 100% [1] with the vast majority of these infections rated as early stage and the fish asymptomatic.

This trout and salmon disease is caused by a myxosporean parasite originating from freshwater bryozoans, *Tetracapsuloides bryosalmonae* (Canning et al. 2002). Relatively short exposures (10 min), to ruptured bryozoans infected with *T. bryosalmonae* results in the invasion of skin mucus cells and produced clinical infections within 8 weeks (Feist et al. 2001, Longshaw et al. 2002). The progressive kidney inflammation and associated hypoplastic anemia is likely to reduce the fitness and performance of affected fish (Clifton-Hadley et al. 1987b). Infections with *T. bryosalmonae* have been detected in natural juvenile Chinook salmon collected in the Merced and Tuolumne rivers [2]. The bryozoan *Fredericella* is reported as a host for *T. bryosalmonae* and was observed at the water intakes of MRH (Okamura and Wood 2002). These authors speculate that salmonid fish may be an accidental host for this bryozoan parasite given the strong inflammatory response characterized by PKD and the observation that infections can occur from water supplies without fish. The recent observations of mature malacosporean spores, that react with DNA and antibody probes to *T. bryosalmononae*, in the urine of rainbow trout with or recovering from PKD suggests that salmonids may indeed be true hosts for the parasite and source of infection for bryozoans (Hedrick et al. 2004). Spores of *T. bryosalmonae* have been shown to be thin walled and fragile with the absence of harden valves as a key characteristic of the newly established order Malacosporea (Canning et al. 2000). Spore infectivity did not exceed 24 h in a study by De Kinkelin et al. (2002) and suggested that reducing byrozoan habitat directly upstream of fish culture facilities could be a viable disease management strategy.

The objective of this study was to follow the health status and performance capabilities of *T. byrosalmonae* -infected MRH juvenile Chinook salmon used for the Vernalis Adaptive Management Plan (VAMP) out-migrant salmon study. These fish were reared at temperatures similar to the San Joaquin River at the California-Nevada Fish Health Center wet laboratory for a period of time that encompassed the out-migration of the VAMP study population.

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[1]Harmon R., K. Nichols, and J.S. Foott. 2004. FY 2004 Investigational Report: Health and Physiological Assessment of VAMP Release Groups–2004. U.S. Fish and Wildlife Service, California-Nevada Fish Health Center, Anderson, CA (report at http://fws.gov/canvfhc).

[2]Nichols K. and J.S. Foott. 2002. Health monitoring of hatchery and natural fall-run Chinook salmon juveniles in the San Joaquin River and tributaries, April – June 2001. U.S. Fish and Wildlife Service, California-Nevada Fish Health Center, Anderson, CA (report at http://fws.gov/canvfhc).

METHODS

Chipp's Island Trawl Kidney Imprints

A sub-sample of 97 adipose fin marked juvenile Chinook salmon collected in the Chipp's Island trawl between 5 May and 27 May 2005 were sampled for kidney tissue by Stockton Fish and Wildlife Office biologists. Imprints of the kidney from 43 salmon with VAMP tag codes were later screened by an indirect fluorescent lectin assay utilizing biotin-labeled *Griffonia simplificola* agglutinin I lectin (GS-I) and fluorescien-labeled avidin stain (Hedrick et al. 1992). The imprints were examined at 40x magnification with an Olympus BHS fluorescent microscope.

Fish Handling

On 25 April 2005, a total of 282 juvenile fall-run Chinook salmon reared at the California Department of Fish and Game's Merced River Hatchery (MRH) was transported to the Fish Health Center Wet Laboratory. Equal numbers of fish were removed from coded wire tagged lots used in the two release groups of the VAMP study. The salmon were held in a 470-L circular tank supplied with aeration. Water temperature was maintained in the recirculation system by immersion heaters within a 300- L effluent sump. Makeup inflow for the system was set at 58 L / min. Water temperature was monitored every 2 h with Onset™ Stowaway temperature loggers. Daily mean water temperatures along the San Joaquin River migration route (Mossdale and Antioch, Fig. 1) were obtained from a California Department of Water Resources real-time website (http://cdec.water.ca.gov). For each of the two releases in May (Table 1), tagged MRH Chinook were placed into the San Joaquin River at Durham Ferry, Dos Reis, and Jersey Point (Fig. 1). A commercial salmon diet was fed at 1.5% body weight per day. The laboratory effluent was treated with 1-2 mg / L chlorine for a minimum of 50 min and discharged into a 1.3 - ha abatement pond.

Necropsy

Fish were captured by net and immediately euthanized in an overdose of MS222, measured for fork length and weight, and examined for pale gill (clinical sign of anemia). Fulton condition factor was calculated from the fork length (KFL = weight / (fork length)^3 x 10^5). The caudal peduncle was cut and blood collected into heparinized microhematocrit tubes within 45 s of capture. Sub-samples of blood were used to prepare a blood smear and a 2.5 µL aliquot that was frozen for later hemoglobin measurement. The microhematocrit tubes were centrifuged at 10,000 x g for 5 min and the packed red cell (hematocrit), buffy coat (leukocrit), and total fluid length was measured with a 30x

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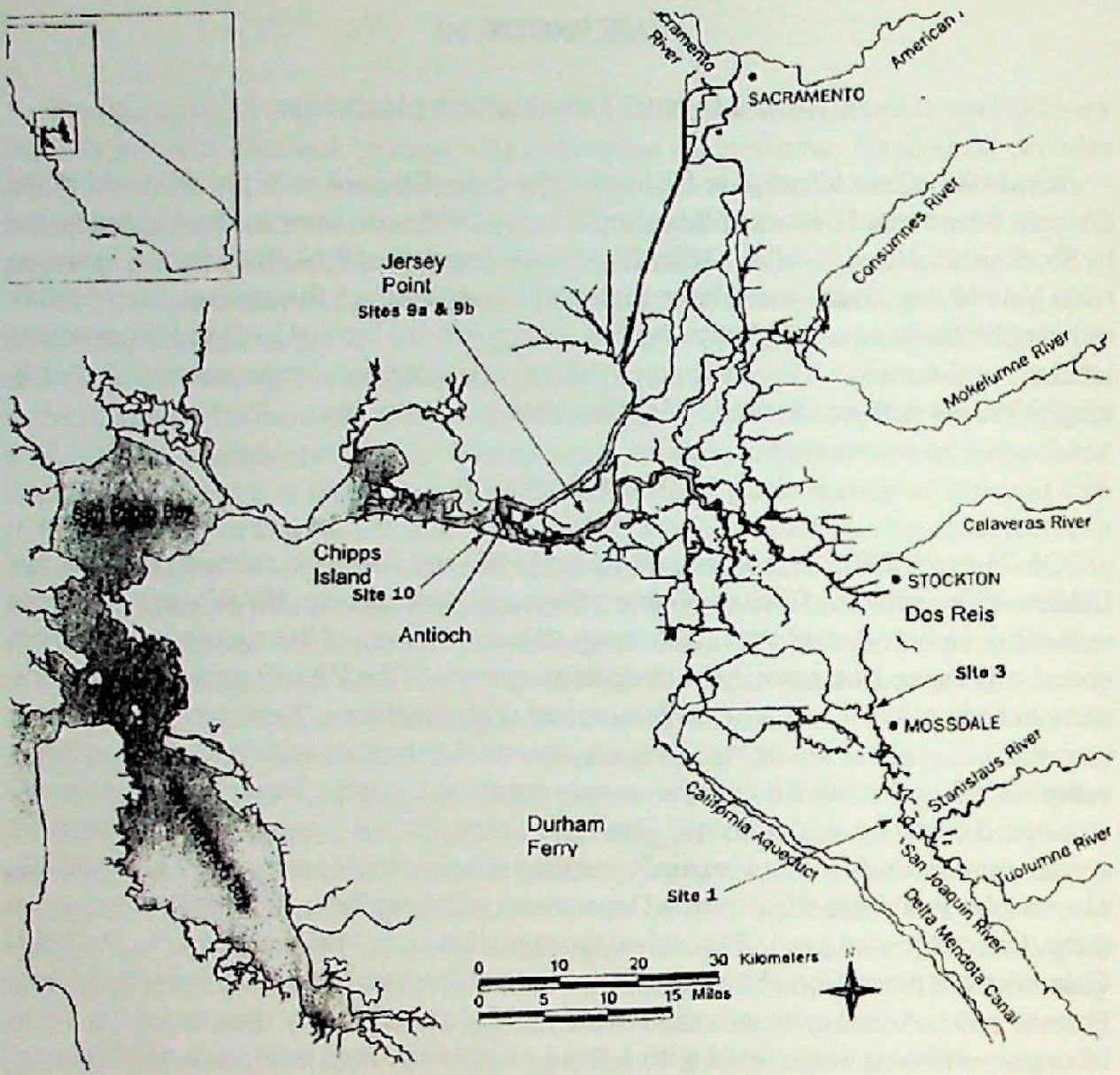

Figure 1. Map of San Joaquin River release sites (Durham Ferry, Dois Reis, and Jersey Point) and trawl collection (Chipps Island) of juvenile Chinook salmon from Merced River Hatchery used for the 2005 Vernalis Adaptive Management Plan study. Temperature measurements along migration route occurred at Mossdale and Antioch.

dissection scope equipped with an ocular micrometer for calculation of both hematocrit and leukocrit (McLeay and Gordon 1977). The mean corpuscular hemoglobin concentration (MCHC), a measure of the average erythrocyte hemoglobin concentration, was calculated: MCHC (g / dL) = [Hb (g/dL) / HCT (%)] x 100. Plasma was frozen on dry ice and stored at -70°C until assayed with colorimetric kits (Pointe Scientific Inc., Lincoln Park, MI) for total protein, glucose, and magnesium. Upon dissection, the degree of spleen and kidney swelling was recorded for each fish. Anterior kidney was collected for a nitroblue tetrazolium (NBT) assay and the posterior kidney was placed

Table 1. VAMP study release sites, dates of release (REL) and last coded wire tag (L-CWT) recovery of juvenile MRH Chinook salmon in the Chipp's Island trawl, incidence of *Tetracapsuloides bryosalmonae* infection in kidney imprints (Tb, no. positive / total examined) and maximum days at large (MDAL) value for each of the 2 release groups.

	Group 1				Group 2			
Site	REL	L- CWT	Tb	MDAL	REL	L-CWT	Tb	MDAL
Durham Ferry (114 rkm)	2 May	19 May	4/4	17	9 May	27 May	2/3	18
Dos Reis (82 rkm)	3 May	12 May	2/3	9	10 May	18 May	1/1	8
Jersey Point (8 rkm)	6 May	15 May	15/18	9	13 May	20 May	1/13	7

in Davidson's fixative, processed for 5 µm paraffin sections and stained with hematoxylin and eosin. On 25 April, kidney – spleen tissue from 60 fish at MRH were collected for viral assays (12, 5 –fish pool samples inoculated onto EPC and CHSE214 cell lines and incubated at 15°C for 18 d). Also *Renibacterium salmoninarum* Direct Fluorescent Antibody Tests were performed on kidney imprints, and kidney inoculum was placed onto Brain Heart Infusion agar for bacteria isolation. Lastly posterior kidneys from 16 MRH fish were collected for histological examination.

Nitroblue Tetrazolium (NBT) Assay

The respiratory burst activity of anterior kidney cell phagocytes was measured in a NBT assay by the method of Secombes (1991). One killing mechanism of activated phagocytes is the production of reactive oxygen species (O_2^-) and is referred to as respiratory burst (Slauson and Cooper 1982). Briefly, the anterior kidney was dissected from a sub-sample of six to eight salmon, weighed to the nearest 0.01 g, placed into cold Hanks Buffered Salt Solution (HBSS), titerated to form a single cell suspension with a 21G needle mounted onto a 1 cc syringe, after a 10µL sub-sample was fixed in Rees-Eckert solution for hemocytometer cell count the suspension was centrifuged (400x g, 4°C, 5 min) and the cell pellet re-suspended in 500 µL of HBSS without calcium or magnesium. One hundred microliters of the cell suspension was added to triplicate wells in a 96-well plate followed by 100 µL of NBT solution (1 mg/mL in HBSS with 1µg/mL Phorbol Myristate Acetate). The reaction was stopped after 20 min incubation at 25°C by centrifuging the plate (400x g, 4°C, 5 min). The supernatant was carefully poured off and the cell pellet fixed by repeat washing with 70% methanol and centrifugation. The cell pellet was air dried and NBT solubilized by the addition of 120µL 2M KOH and 140 µL DMSO. The optical density of the solution was measured at 630 um in a Biotek microplate reader. The data was expressed as mOD630 per 10^5 anterior kidney cells. In all cases, the processing of the anterior kidney cells began within 2 h of collection.

Saltwater Challenge –

Groups of six fish were held for 24 h in 19-L buckets containing 25 – 26 ppt saltwater (Instant Ocean aquarium salt mix) supplied with aeration and held in a water bath set at 13 - 14°C. After 24 h, all fish were rapidly netted and euthanized with an overdose of MS222 in saltwater, gently dried, weighed to the nearest 0.1 g and the fork length measured (mm), bled into a heparinized microhematocrit tube from the severed caudal peducle, and gill lamellae placed into Sucrose-EDTA-Imidazole buffer and frozen at -70°C. Gill Sodium- Potassium - Adenosine Triphosphatase activity (ATPase = µmoles ADP / mg protein / h) was assayed by the method of McCormick and Bern (1989). After centrifugation, hematocrit and leukocrit were recorded for each blood sample. Plasma was frozen for later sodium (flame photometer), magnesium and total protein measurements (colorimetric assays).

PKD Score

The entire kidney section was scored a 0 - 3 for the relative number of parasites (0=none, 1 =< 10, 2 = 11 - 30, 3 = > 30) and degree of inflammation (0 = none, 1 =< 10% of kidney, 2 = 11 – 50%, and 3 = > 50%). If the fish's hematocrit was less than 20%, a score of 6 was assigned to the fish. An individual's PKD score was the summation of the degree of anemia (hematocrit score of either 0 or 6), parasite load (2 multiplied by the parasite number value), and degree of inflammation (3 x inflammation value). PKD scores ranged from 0 (normal) to 24 (late stage clinical disease). PKD scores were used to sort fish for statistical analysis.

Swim Exhaustion Performance –

Swimming performance was determined by the amount of time a given fish could swim at a relatively high velocity before becoming exhausted (Jones and Moffitt 2004). Groups of 5 - 10 salmon were placed into a 30-cm diameter x 56 - cm length cylinder and allowed to acclimate for 20 min to a minimal 18.3 cm/s flow generated by an electric trolling motor. This rate equated to approximately two body lengths/s (BL/s) and was close to the reported optimal 1 BL/s cruising speed for salmonids (Webb 1995). The ends of the cylinder were enclosed with 1-cm honeycomb screens to provide uniform flow pattern. The upstream half of the chamber was covered to encourage fish to swim in this region and an open hatch at lower end allowed for observation and capture of impinged fish. The entire cylinder was held in a 1400-L oval tank with a partial middle wall for circular flow. Water was kept at the same temperature as the rearing tank. Flow was measured in front of the rear screen by a Flowmate Model 2000 flowmeter (Marsh-McBirney Inc., Maryland). After acclimation, the flow was increased to 48.8 cm/sec for an additional 100 min. If a fish became impinged on the rear screen of the chamber, it was prodded to induce it to return to the upper portion of the chamber. Any fish that remained on the posterior screen was considered to be exhausted, the time recorded, and the fish was sampled in a manner similar to the weekly physiological group (weight,

length, blood, and kidney histology). After 2 h, all remaining fish were captured and sampled as stated above.

Statistical Analysis

For data sets that were not normally distributed, Kruskal-Wallis 1-ANOVA on ranks along with Dunn's Multiple comparison method was used to compare significance among raw sample group data and is reported as an "H" value.

RESULTS

Relationship to VAMP Tagged Fish Recovery and Out-Migration

Salmon were brought to the wet lab 6 days prior to the first VAMP release at Durham Ferry on 2 May and reared for 50 d (Table 1). Daily mean water temperature of the study fish tank ranged from 14.5 - 19.6° C and was similar to temperatures at Mossdale but averaged 2 °C lower than the San Joaquin River at Antioch (Fig. 2). The Mossdale gauge was chosen to be reflective of the Durham Ferry and Dos Reis release sites, while the Antioch gauge data should approximate the final capture location at Chipp's Island.

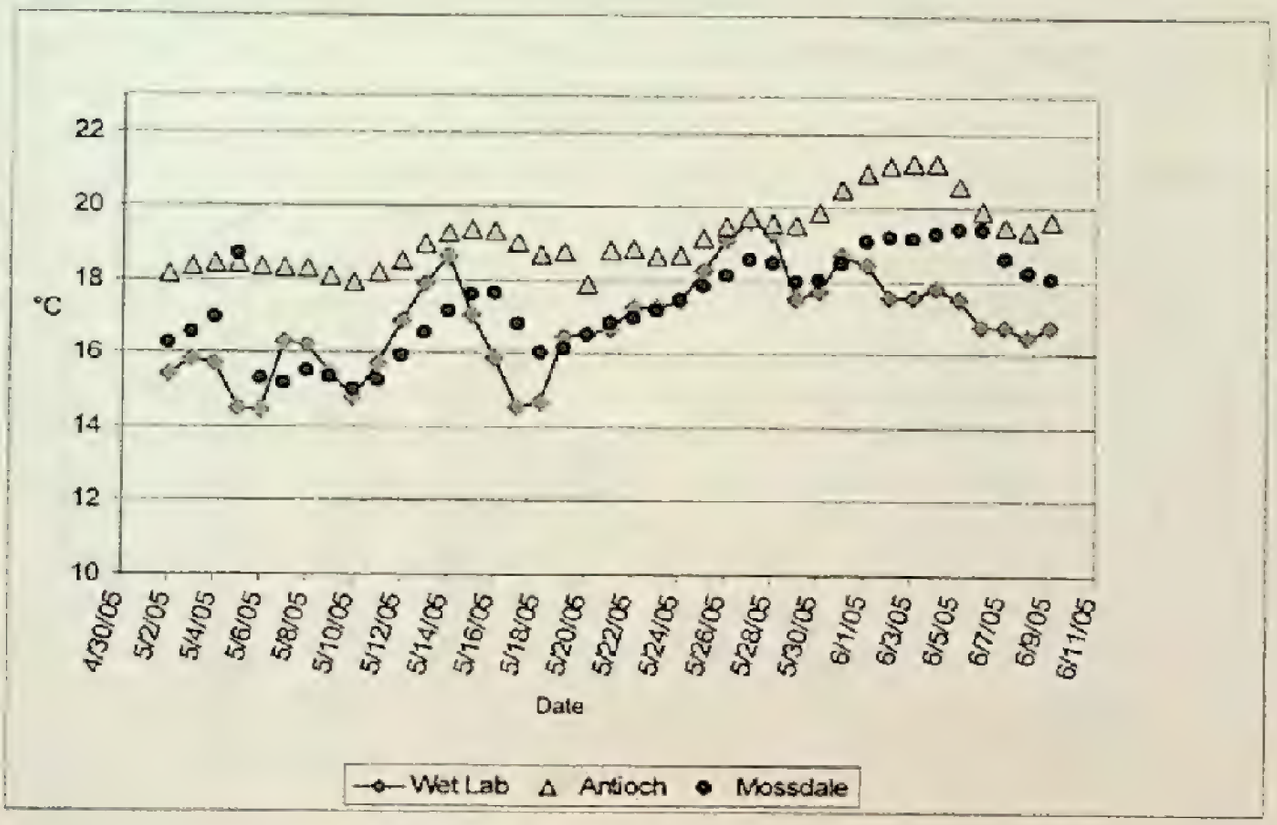

Figure 2. Mean daily water temperature profile (°C) for juvenile Chinook salmon from Merced River Hatchery held at the wet lab compared to profiles from two locations along the San Joaquin River smolt migration route (Mossdale and Antioch).

The parasite, *T. bryosalmonae,* was observed in 40% (17 of 43) of the VAMP tag salmon kidney imprints collected from the Chipp's Island trawl (Table 1). It is unclear why the second Jersey Point group had such a low incidence (1 of 13 [8%]) in comparison to the other groups (67 – 100%). The number of parasites per imprint was highly variable and ranged from 1 to over 100, with a mean of 13 (std. dev. 21). Neither virus nor *Renibacterium salmoninarum* was detected in the MRH population inspected on 25 April. *Aeromonas hydrophilia* was isolated in 2 of 42 bacterial cultures and 6 of the 16 histological samples of kidney contained very low numbers of *T. bryosalmonae,* without associated inflammation.

Proliferative Kidney Disease (PKD)

The level of clinical disease, as portrayed by the PKD score, markedly increased starting with the 29 days post-transfer (dpt) from MRH sample (Fig. 3). A severe disease state occurred in the study population after the last VAMP coded wire tag salmon was captured at Chipp's Island on 27 May. A total of 76 study salmon (27% cumulative mortality) died due to PKD beginning at 36 dpt (Fig 3). No secondary infections were detected in the 18 mortalities examined for external parasites and cultured for systemic bacteria. Clinical signs such as swollen kidney and spleen as well as pale gill were observed in the study fish beginning at 36 dpt (33% of sample) and became more prevalent (88-100% of sample) with time. Late stage PKD was histologically characterized by hyperplasia and granuloma formation in the kidney interstitium and large numbers of *T. bryosalmonae* trophozoites.

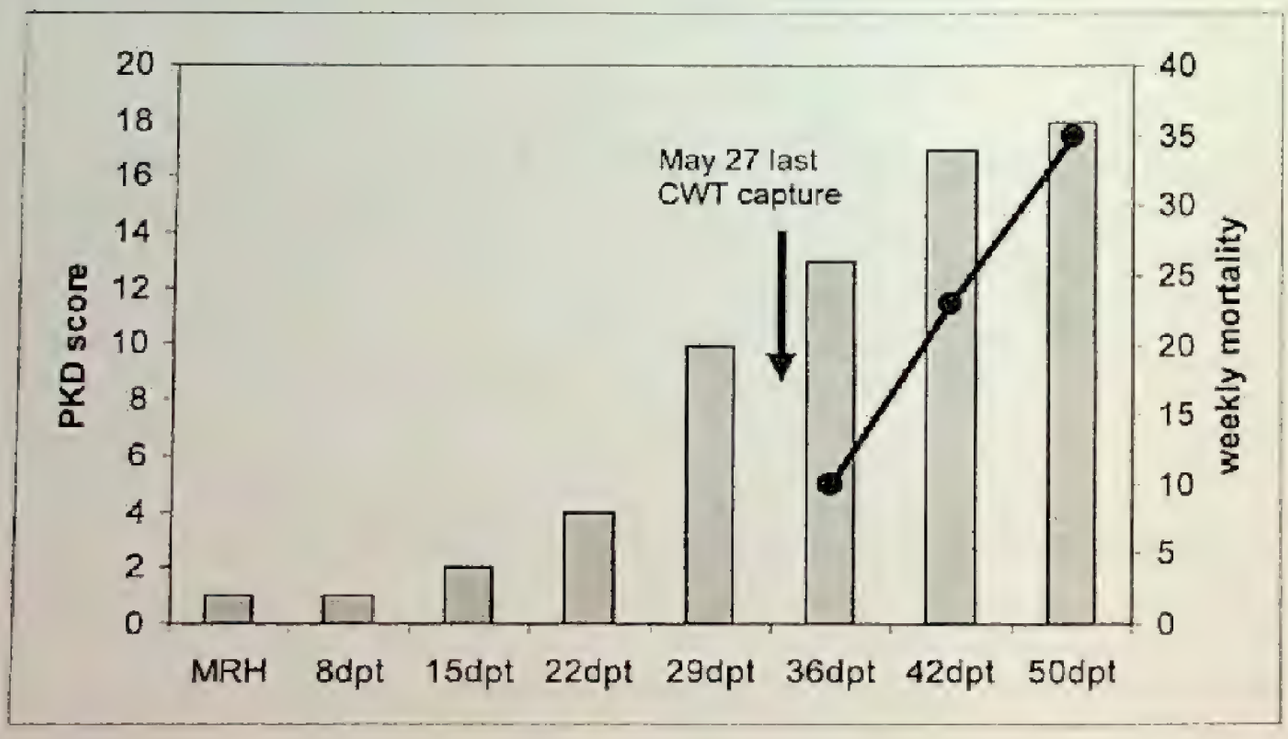

Figure 3. Mean PKD score for juvenile Chinook salmon from Merced River Hatchery (MRH) sampled at the hatchery and each week up to 50 days post-transfer (dpt). Weekly mortality is also displayed as number of salmon that died of PKD over 7 d period.

Morphological and Physiological Characteristics –

Minimal growth occurred in the study group over the 7-week period. Mean weight ranged from 5.5 to 6.8 g, while mean fork length ranged from 81 to 86 mm. Condition factor remained similar throughout the study, with mean values ranging between 0.990 and 1.157. As estimated by hematocrit and leukocrit, erythrocyte numbers declined while white blood cell numbers increased,starting at 42 dpt (Table 2, Fig. 4). The hematocrit of the MRH and 8 dpt sample groups were significantly higher than 29 – 50 dpt groups ($H = 64.3$, 7df, $p<0.001$). Anemia was apparent by the observation of pale gills in 88 and 100% of fish at 42 and 50 dpt, respectively. The percentage of bloodsmears with > 1% of immature erythrocyte jumped from 0% to >60% beginning at 42 dpt (Table 2). Both time and disease state correlated with reduced hematocrit and increased leukocrit. Linear regression analysis of hematocrit with sample time (dpt) and disease state (PKD score) demonstrated a R^2 of 0.66 and 0.58, respectively. Similarly, increased leukocrit values were moderately correlated with PKD score ($R^2 = 0.3$) and dpt ($R^2 = 0.42$). The leukocrit of the MRH and 8 dpt sample groups were significantly lower than 36 – 50 dpt groups ($H = 44.8$, 7df, $p<0.001$). Hemoglobin concentration and MCHC index declined after the 36 dpt sample group and were at their lowest points in the 42 dpt sample (Table 2). The MCHC of the 42 dpt group was significantly lower than MRH, 8, 15, and 29 dpt groups ($H = 38.1$, 7df, $p<0.001$). Days post-transfer was better correlated with MCHC decline ($R^2 = 0.21$) than PKD score ($R^2 = 0.15$). The white blood cell profile did not change to any great degree over the 7-weeks study, with lymphocytes being the dominant cell type (Table 2). Both the highest number of cells per gram of anterior kidney and the greatest NBT activity was observed at 36 dpt (Table 2). There was no correlation between PKD score and NBT reaction ($R^2 = 0.001$).

Plasma protein concentrations of salmon increased beginning with the 29 dpt sample group. A maximum mean value of 3.8 g / dL occurred in the 42 dpt fish (Fig. 5). Similarly, plasma protein levels in 42 and 50 dpt swim endurance fish were also elevated in comparison to the previous weeks (Table 3). The correlation between PKD score and increased plasma protein concentration was relatively low ($R^2 = 0.17$). Plasma glucose values remained relatively low (< 90 g / dL), indicating that sampling occurred prior to a secondary stress response. In contrast, swim challenge fish tended to have higher plasma glucose than the unstressed cohorts (Table 3). Mean glucose values of 25 and 41 g / dL for the 50 and 29 dpt sample groups were significantly lower than the MRH, 8, and 42 dpt groups ($H = 47.7$, 7df, $p<0.001$). Similarly, glucose values for the 42 and 50 dpt swim challenge fish were significantly lower than 8 and 22 dpt groups ($H = 28.4$, 6df, $p<0.001$). Plasma magnesium values decline over time (Table 3, Fig. 6). This decline was somewhat correlated to PKD score ($R^2 = 0.33$). The 29, 42, and 50 dpt fish had significantly lower plasma magnesium values than MRH and 8 dpt groups ($H = 48.9$, 7df, $P<0.001$). Swim challenge fish showed a similar declining plasma magnesium trend.

Table 2. Blood cell characteristics and anterior kidney phagocytic activity of Juvenile MRH Chinook salmon sample at the hatchery and from 8 to 50 days post-transfer at the wet lab. Mean (std. dev.) values of hematocrit (%, HCT), hemoglobin concentration (g / dL, Hb), mean corpuscular hemoglobin concentration (g / dL, MCHC), leukocrit (%, LCT), differential leukocyte count percentage of lymphocytes, thrombocytes, and neutrophils, the prevalence of bloodsmears containing > 1% immature erythrocytes (>1% IE), reduction of nitroblue tetrazolium dye (mOD/ 10^5 cell) by the respiratory burst of activated anterior kidney cells (NBT), and the concentration of cells per gram of anterior kidney (AK cell / g x 10^8) in sample groups.

	MRH	8dpt	15dpt	22dpt	29dpt	36dpt	42dpt	50dpt
HCT	44 (3)	45 (6)	39 (4)	38 (5)	35 (5)	35 (5)	22 (3)	21(6)
Hb	7.5 (0.6)	7.4 (1.1)	6.5 (1.0)	5.8 (1.3)	6.6 (0.7)	4.7 (0.7)	1.7 (0.4)	2.5 (1.1)
MCHC	17 (1)	17 (3)	17 (2)	16 (5)	20 (3)	14 (3)	8 (2)	12 (4)
LCT	0.498 (0.167)	0.607 (0.177)	0.773 (0.133)	0.977 (0.231)	0.800 (0.192)	1.429 (0.808)	2.693 (1.00)	2.968 (2.336)
%lymphocyte	71 (16)	78 (12)	82 (10)	86 (9)	87 (7)	90 (4)	86 (12)	88 (5)
%thrombocyte	20 (16)	21 (12)	16 (10)	12 (8)	11 (3)	10 (4)	13 (11)	12 (5)
%neutrophil	3 (4)	0	2 (3)	2 (2)	0	0	0	1 (1)
> 1% IE	0 / 5 (0%)	0 / 5 (0%)	0 / 5 (0%)	0 / 5 (0%)	0 / 5 (0%)	0 / 5 (0%)	3 / 5 (60%)	4 / 5 (80%)
NBT	ND	24 (8)	12 (5)	23 (8)	14 (4)	34 (12)	18 (3)	16 (5)
AK cell /g (10^8)	ND	9.4 (2.7)	8.6 (4.2)	8.3 (1.2)	11.8 (2.5)	15.4 (7.9)	ND**	10.7 (4.5)

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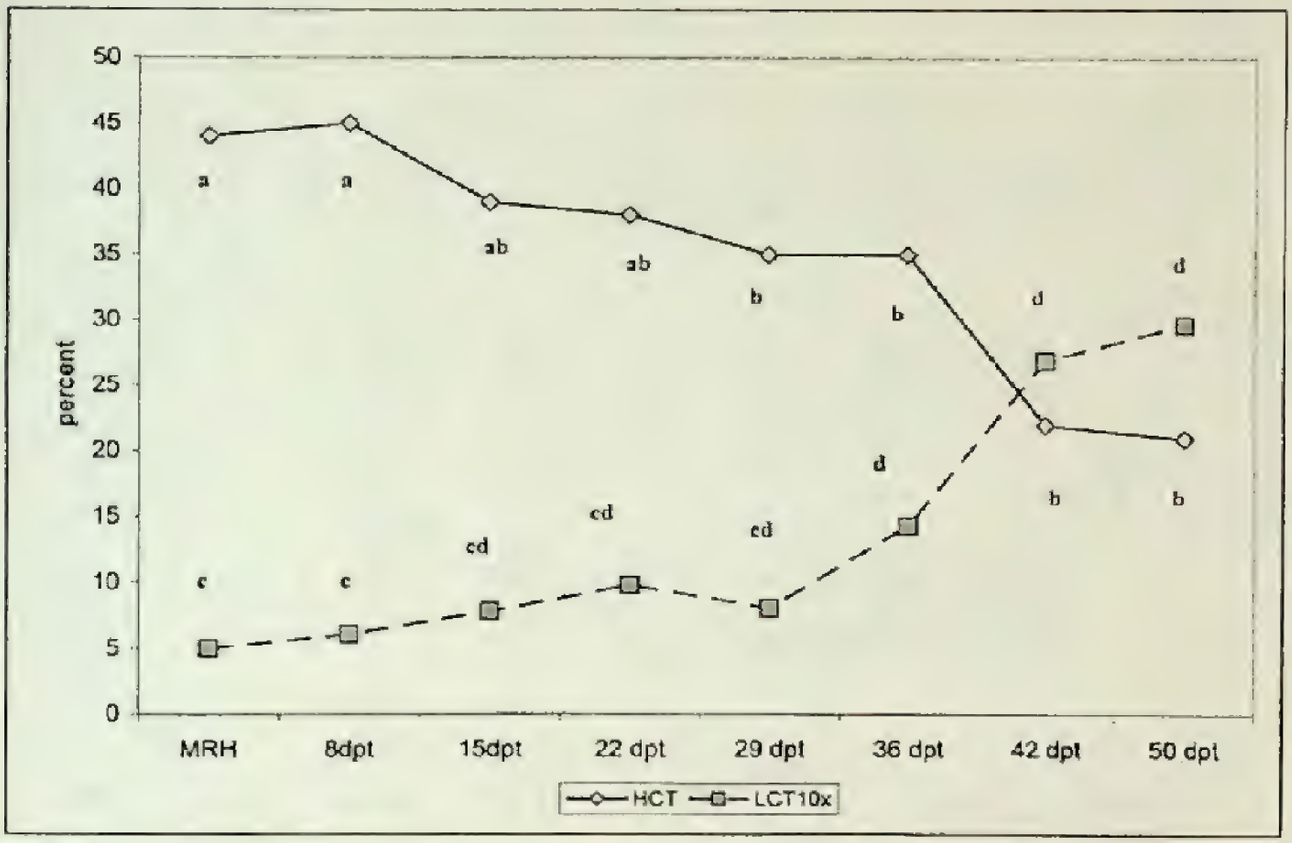


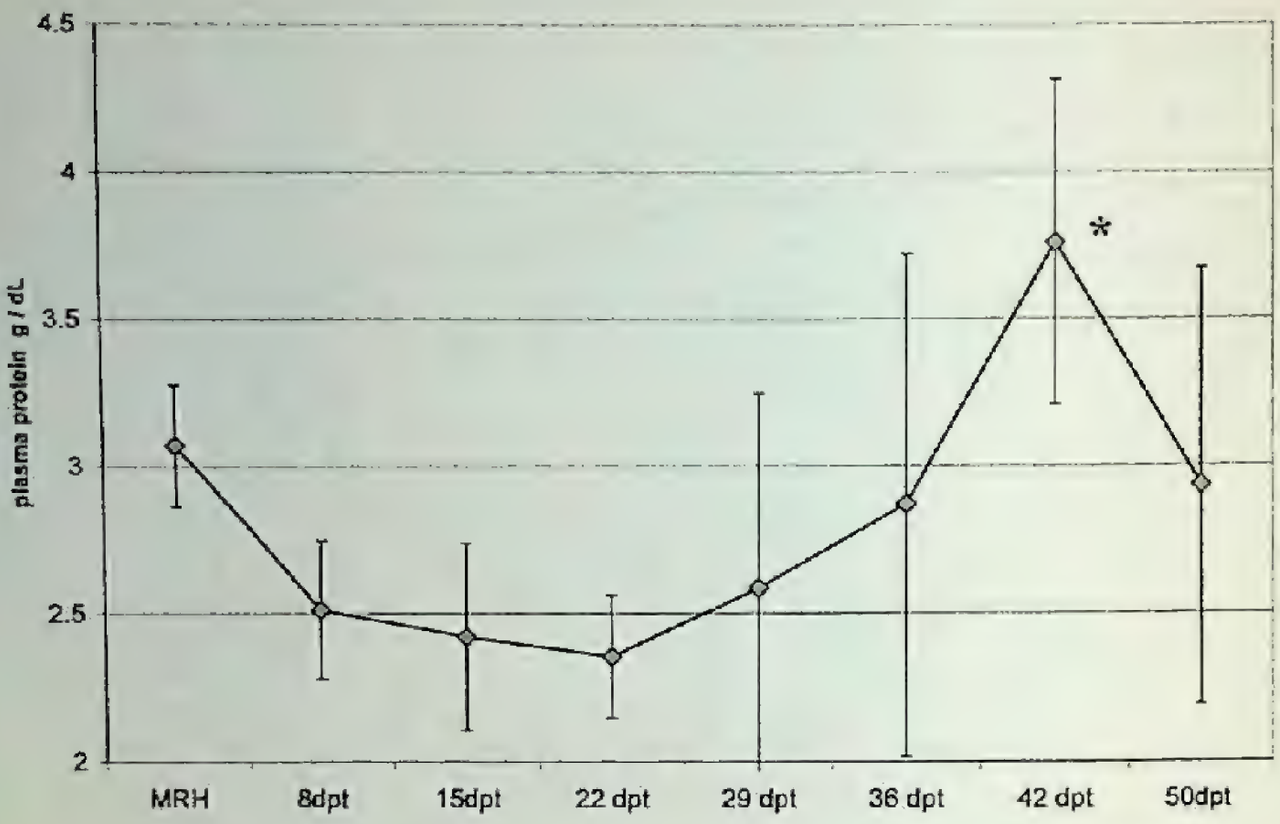
Figure 4. Mean hematocrit (HCT) and 10x leukocrit (LCT10x) values of juvenile Chinook salmon from Merced River Hatchery held at the wet lab. Mean values [(a) and (b) for hematocrit and (c) and (d) for leukocrit] with different subscripts are significantly different ($P < 0.05$).

Swim Endurance

There was no observed PKD effect on the exhaustion swim challenge performance until 50 dpt (Table 4). Five of seven fish in the 50 dpt challenge did not complete the 120 - min test. Lower hematocrit values in the 50 dpt fish were weakly correlated with endurance time ($R^2 = 0.18$). The markedly lower hematocrit values and high PKD scores observed in the 42 dpt fish did not correspond to a drop in their swimming performance.

Saltwater Adaptation

No fish died during the 24-h saltwater (SW) challenges performed between 22 and 50 dpt. Smolt development measurements indicated that the study fish were in an advanced stage of smoltification. Saltwater adaptation performance did not decline until at 50 dpt (Table 5). Four 50 dpt salmon had plasma sodium levels in excess of normal values (> 170 mmol/L) and the mean percent of normal FW condition factor fell to 87% for the group. Lower condition factors of SW fish indicate dehydration due to poor ion regulation. The degree of anemia, as measured by declining hematocrit, was poorly correlated with elevated plasma sodium concentrations ($R^2 = 0.11$). As with the FW



igure 5. Mean (std. dev.) plasma protein concentrations (g / dL) of juvenile Chinook salmon .om Merced River Hatchery held at the wet lab study fish . Asterisk denotes statistical difference from all other groups.

sample groups, plasma protein levels became elevated starting in the 42-dpt sample group but were only weakly correlated with PKD score ($R^2 = 0.12$). Plasma protein levels of fish with PKD scores > 12 were significantly higher than cohorts with lower PKD scores (Mann-Whitney Rank Sum test, P<001). No difference in plasma magnesium concentration was observed between the groups (H= 5.48, 4df, P=0.241). Magnesium concentration was not correlated with PKD score ($R^2 = 0.009$). The decline in gill Na-K-ATPase activities with time was not considered biologically significant (H=12.61, 4 df, P=0.016) and was poorly correlated with PKD score ($R^2 = 0.09$). It should be noted that the 29-dpt challenge occurred near the date of the last Chipp's Island CWT recovery and that the 29-dpt fish perform quite well.

DISCUSSION

Merced R. Hatchery Chinook smolts had a high incidence of *T. bryosalmonae* infection in 2005. Kidney imprints were collected from 73% (43 of 59) of the total VAMP-tag salmon collected at Chipp's Island trawl and *T. bryosalmonae* was detected in 40% of these samples. The prevalence of *T. bryosalmonae* infection ranged from 38% in the 25April MRH inspection to 100% by 22 dpt to the wet laboratory. Since 2000, the incidence of *T. bryosalmonae* infection in MRH salmon juveniles has ranged from 4 –

Table 3. Mean (std.dev.) values and sample numbers (n) for plasma protein(TP g/dL) , glucose (GLU g/dL), and magnesium (Mg mEq/L) of juvenile salmon sampled at Merced R. hatchery (MRH) and at 8 to 50 days post transfer (dpt). Similar data reported from swim challenge salmon (swim). Means in the same row with different subscripts are statistically different (P<0.05).

	MRH	8 dpt	15 dpt	22 dpt	29 dpt	36 dpt	42 dpt	50 dpt
TP	3.1 (0.2) n=15 ab	2.5 (0.2) n=14 a	2.4 (0.3) n=15 a	4.2 (0.2) n= 8 a	2.6 (0.7) n=10 a	2.9 (0.9) n = 12 ab	3.8 (0.6) n = 8 b	2.9 (0.7) n=5 ab
TPswim	ND	2.6 (0.3) n=10 ab	2.5 (0.3) n=5 ab	2.5 (0.1) n = 5 ab	2.2 (0.2) n= 5 b	3.0 (0.4) n=9 ab	3.1 (0.3) n = 9 a	3.6 (1.1) n=7 a
GLU	69 (10) n=15 a	65 (8) n=13 ac	57 (16) n=15 abc	47 (7) n=8 abc	41 (9) n=10 b	61 (53) n=12 bc	70 (24) n=8 ac	25 (4) n=4 b
GLU swim	ND	116 (26) n=10 a	69 (18) n=5 ab	131 (15) n=4 a	48 (9) n=5 ab	75 (33) n=9 ab	39 (25) n=9 b	50 (48) n=7 b
Mg	2.6 (0.2) n=15 a	2.5 (0.4) n=14 ac	2.1 (0.4) n=15 abc	2.0 (0.6) n=8 abc	1.6 (0.3) n=10 b	2.0 (0.4) n=10 bc	1.7 (0.3) n=8 b	1.5 (0.2) n=4 b
Mg swim	ND	2.3 (0.3) n=10 a	2.0 (0.3) n=5 ab	1.5 (0.6) n=4 ab	1.7 (0.1) n=5 ab	2.0 (0.4) n=9 ab	1.5 (0.3) n=9 b	1.5 (0.4) n= 7 ab

ND = not done

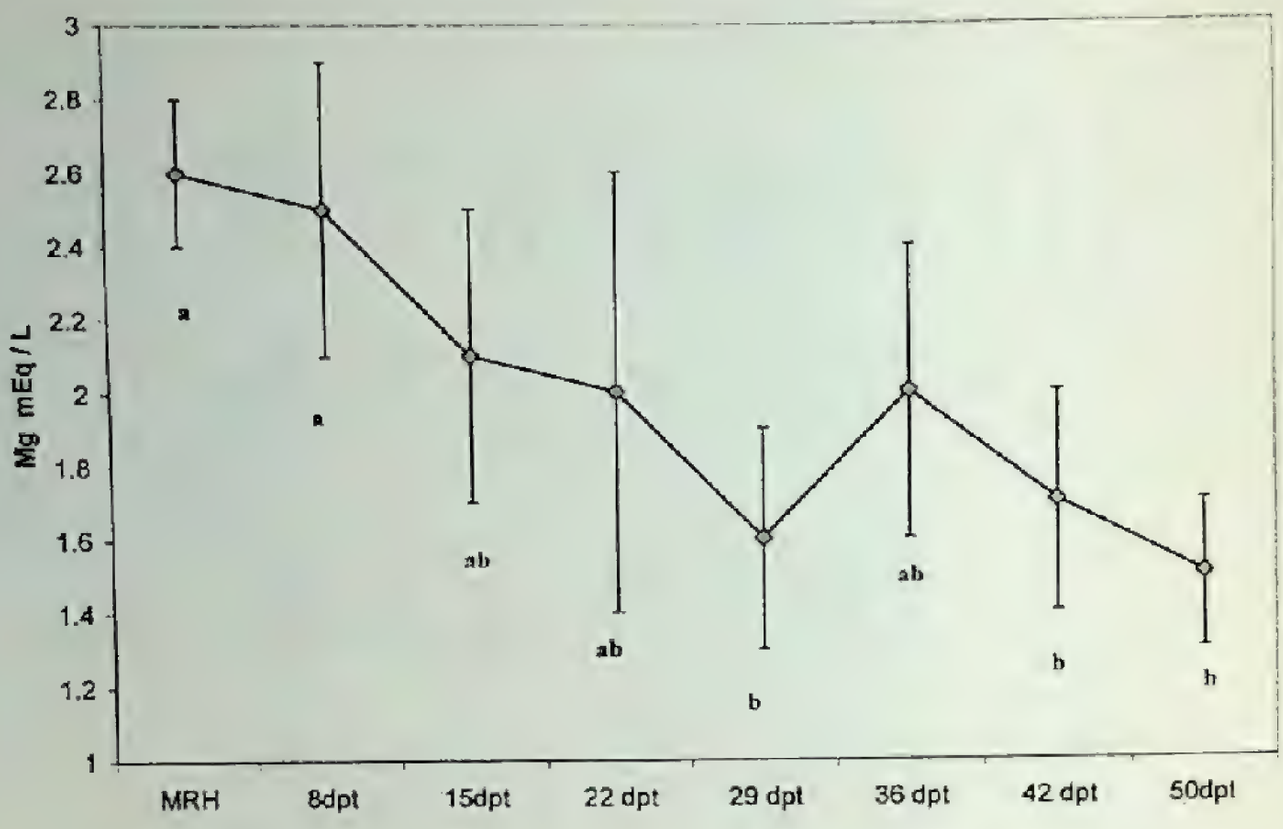


Figure 6. Mean plasma magnesium concentration (mEq / L) of juvenile Chinook salmon from Merced River Hatchery held at the wet lab. Letters denote statistically difference (P<0.001) and bars are standard deviation.

Table 4. Swim exhaustion data for juvenile MRH Chinook salmon challenged each week up to 50 days post-transfer (dpt). Mean (std. dev.) for Body length / sec at maximum flow (max BL / s), PKD score, and hematocrit (HCT). Also reported is the percent of test group that became exhausted (% exhaust) before 120 min. and the range of exhaustion times in minutes (Times).

	8 dpt	15 dpt	22 dpt	29 dpt	36 dpt	42 dpt	50 dpt
Max BL/s	3.3 (0.1)	6.0 (0.3)	5.9 (0.2)	5.9 (0.2)	6.0 (0.4)	5.7 (0.4)	7.9 (0.4)
% exhaust	0	0	0	0	0	0	71
Times (min-max)	na	na	na	na	na	na	41 - 82
PKD score	1	2	3	11	12	17	19
HCT	47 (5)	63 (6)	39 (6)	36 (3)	32 (5)	25 (4)	27 (8)

na not applicable

Table 5. Smolt development measurements for juvenile Merced River Hatchery (MRH) Chinook salmon sampled at the hatchery and up to 50 days post transfer (dpt) to wet lab. Percent of mean FW cohort condition factor (%FW KFL), plasma sodium (mmol / L), plasma protein (g/dL), plasma magnesium (mEq/L), number of salmon in SW challenge and their gill sodium-potassium adenosine triphosphatase activity (mmoles ADP / mg protein / hr) data reported as mean (std. dev.). PKD score for challenge group reported as median value.

	MRH	22dpt	29dpt	36dpt	42dpt5	50dpt
MedianPKD	0	4	8	15	16	18
%FWKFL	n/a	91 (4)	96 (6)	100 (8)	100 (8)	87 (7)
PlasmaSodium	n/a	139 (12)	154 (7)	156 (13)	153 (10)	170 (9)
PlasmaProtein	n/a	2.3 (0.2)	2.4 (0.6)	2.4 (0.2)	3.1 (0.4)	3.5 (0.6)
PlasmaMg	n/a	4.0 (2.5)	3.3 (1.9)	3.9 (2.0)	3.0 (0.7)	2.9 (0.8)
Fish in SW chall.	n/a	10	12	12	32	12
Gill Na-K-ATPase	10.5 (2.3) N = 12	11.2 (3.9) N = 9	10.5 (2.9) N = 12	9.5 (2.1) N = 12	7.2 (3.3) N = 32	7.0 (3.3) N = 7

n/a = not applicable as no SW challenge performed

100%[3]. These fish have been sampled in early May and the majority of these infections have been rated as moderate, with minimal kidney swelling.

At the time of the last VAMP-tag recovery from the Chipp's Island trawl on 27 May, the degree of PKD was judged to be relatively mild in the study fish. During the spring smolt migration, water temperatures in the Delta can be in excess of 18°C and are thus conducive to the rapid expression of PKD (Foott et al. 1986, Baker et al. 1995). Approximately 3 weeks after the last VAMP fish recovery, MRH salmon held in the laboratory were in a severe disease state, with the study group having a 27% cumulative mortality due to PKD. The severity of PKD was also exemplified by a high degree of kidney inflammation and associated physiological impairment. It is unlikely that PKD significantly influenced tagged fish recovery in 2005; however PKD could be a significant mortality factor for Merced River salmon smolts during their early estuary and ocean entry phase.

In the American River, the seasonal infectivity of *T. bryosalmonae* ranges from April through September (Foott et al. 1986). Given the early stage of the infection in the 25 April MRH sample group, we assume a similar seasonality in the Merced River. Clifton-Hadley et al. (1987a, b) reported that PKD will follow a 20+ week course, with fish eventually recovering from the infection. If we assume that the 50-dpt group was near the peak of the clinical disease stage, then the initial infections probably occurred 10 weeks earlier in late March or early April. As mentioned above, the timing of clinical disease coincides with the period when MRH smolts would be entering San Francisco Bay and the ocean.

The low incidence of light *T. bryosalmonae* infections observed at MRH on 25 April did not indicate that the population would undergo severe PKD in less than 7 weeks. This observation provides a cautionary note to any health predictions made on late April–early May inspections. Clinical signs and morbidity increased with time and were not closely correlated to the current histological scoring system. For instance, anemia was better correlated to dpt ($R^2 = 0.66$) than PKD score ($R2 = 0.58$). In the future, image analysis of kidney sections to determine the degree of interstitial hyperplasia may provide a better tool for the identification of different PKD stages. Conversely, a simpler system using gross renal and splenic swelling categories could be employed as done by Clifton-Hadley et al. (1987a).

The progressive patho-physiological changes observed in this study were consistent with other reports on PKD (Hedrick et al. 1986, Clifton-Hadley et al. 1987b, Foott and Hedrick 1990). The study fish showed signs of anemia and compensatory erythropoiesis by 42 dpt. The co-occurrence of a hematocrit below 20% and pale gill was matched with indicators of compensatory erythropoiesis such as increased numbers of immature erythrocytes in the blood and a reduction in the mean corpuscular hemoglobin content (MCHC). The range of hemoglobin concentration, hematocrit, and MCHC observed in the MRH and 8-22 dpt sample groups were similar to the range described for immature rainbow trout and Coho salmon (Wedemeyer and Chatteron 1971, McCarthy et al. 1975, Miller et al. 1983). Foott and Hedrick (1990) reported that rainbow trout, with asymptomatic PKD but having histological kidney lesions, had MCHC values of 17 in comparison to the 19 of the uninfected controls. The mean MCHC of the study fish did not drop below 16 until 36 dpt and were at 8 and 12 in the 42 and 50 dpt sample groups, respectively. Gallauger and Farrel (1998) state that rainbow trout with hematocrits <20% were considered anemic. We used this value to delineate anemia as it was less than half of the hematocrit values of healthy salmon in the MRH and 8 dpt sample groups. Hematocrit < 20% was also associated with pale gill.

A number of reports have linked reduced swimming performance in salmonid fishes with a disease state (Butler and Milleman 1971, Tierney and Farrell 2004). Swimming performance was not closely correlated with low hematocrit values or PKD score. Despite severe PKD, most of the 42 and 2 of 7 50 dpt salmon could complete the 120 -min swim challenge at rates of > 6 body lengths/sec. Brauner et al. (1993) experimentally reduced the concentration of functional hemoglobin in juvenile Chinook salmon and did not detect a decline in the critical swimming speed (Ucrit) until hemoglobin concentration was < 30 %. These authors hypothesize that other mechanisms could aid fish in maintaining swimming speed (i.e., anaerobic metabolism, increase cardiac output, shift of blood from internal organs to muscle, etc). Further investigation of this performance measure should examine infected smolts later in the course of the disease. The use of hematocrit as a measure of oxygen transport capacity in the blood has been criticized due to the variability imposed by sample technique and that transient deficits in oxygen carrying capacity can be met by changing cardiac output, ventilatory flow, or lamellar perfusion (Houston 1997). The lack of correlation between low hematocrit and swimming performance of the study fish could be explained by the compensatory mechanism mentioned above. We chose hematocrit as our measure of circulating

erythrocyte numbers based on its ease of measurement in comparison to manual erythrocyte counts. Despite it limitations, we feel that hematocrit provided a valid estimate of erythrocyte concentration and could detect anemia in the population.

Kidney inflammation may have impaired divalent ion re-absorption, as evident by the marked decline in plasma magnesium values. This trend was observed in salmon sampled immediately after capture and after 120 min exhaustive swimming challenges. Magnesium levels in the healthy MRH and 8 dpt sample salmon were similar as those reported for normal Lake Trout (Edsall 1999). Complete kidney tubule dysfunction was not apparent in the study fish given their elevated plasma protein profile. Plasma glucose also tended to be within a normal range until 50 dpt. It is possible that insufficient liver glycogen reserves could have contributed to this low glucose level at 50 dpt Feeding was poor during the 42 to 50 dpt period.

The salmon in the study appeared to be responding to *T. bryosalmonae* infection with a strong immune system response. Circulating white blood cell numbers increased steadily during the course of the study. Leukocrit values reached over 2% by 42 dpt: however, we are uncertain on whether the cell density of immature erythrocytes would result in these cells being part of the buffy coat measured for leukocrit. This concern is prompted by the poor correlation between elevated leukocrit and the low numbers of white blood cells seen in corresponding blood smears taken at 42 and 50 dpt. Leucocytosis has been reported in trout affected by PKD (Clifton-Hadley et al. 1987b, Angelidis et al. 1987, Foott and Hedrick 1990, Chilmonczyk et al. 2002). The composition of the white blood cell population remained similar throughout the study with the lymphocytes being the dominant cell type. Chilmonczyk et al. (2002) reported that lymphocyte proliferation was the key cellular change during clinical PKD and is responsible for the renal hyperplasia. These authors also stated that phagocytosis of latex beads by anterior kidney cells declined in trout showing clinical signs of disease. We did not observe a reduction in the reactive oxygen production by stimulated anterior kidney cells in the NBT assay. This observation was true for clinically diseased salmon sampled at 42 and 50 dpt. A similar observation was made by Foott and Hedrick (1990) for NBT response in trout with subclinical PKD. The increased plasma protein levels seen in salmon sampled at 42 dpt could reflect an increase in acute phase proteins as reported in PKD affected trout (Scott 1984, Klontz et al. 1986, Foott and Hedrick 1990).

The MRH salmon were undergoing smoltification during the month of May. High gill ATPase levels and normal ion regulation in the saltwater challenges were seen in the study fish until 50 dpt. It is likely that advanced PKD was responsible for the impaired ion regulation seen in the 50 dpt group. Despite their elevated plasma sodium and signs of dehydration, no mortality occurred in this group during its 24 h SW challenge. Given the severity of PKD observed in the study fish, osmoregulatory success of any MRH salmon entering saltwater after 50 dpt is questionable. The decline in gill Na-K-ATPase activity seen during the study may be a result of elevated water temperature (McCormick et al. 1999). We have observed a similar response to extended rearing in elevated water temperatures in juvenile Chinook from Trinity River Hatchery. In saltwater, there is a marked decrease in urine flow (now isoosmotic to the blood) and an active excretion of the divalent ion magnesium by the kidney tubules (Clarke and

Hirano 1995). Unlike Kent et al. (1994), we did not observe elevated plasma magnesium levels in infected salmon held in SW. Plasma magnesium (decrease) and protein (increase) of the SW fish followed the same trend as their FW cohorts. In summary, no significant kidney impairment for SW adaptation was observed in the study group until 50 dpt. Osmoregulatory performance began to decline at this point in the disease. In future studies, challenging uninfected control fish will provide an insight into any captivity-influenced changes independent of PKD. Proliferative kidney disease has been shown to follow the same disease course in saltwater as in freshwater (Hedrick and Aronstien 1987). The authors noted that mortality was much lower in Chinook salmon juveniles held in the saltwater laboratory system compared to their freshwater cohorts due to the arrest of FW ectoparasites and external bacteria. Merced River smolts moving into the Bay may also benefit from cooler water temperature and effects of salinity on FW ectoparasites.

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SIZE DIFFERENCES IN WILD AND FARMED RED ABALONE: DEVELOPING ENFORCEMENT TOOLS TO COMBAT ILLEGAL COMMERCIALIZATION

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Enforcement tools are needed to combat the illegal commercialization of wild red abalone, *Haliotis rufescens*, taken in the recreational fishery in northern California. We examine size differences between wild and farmed red abalone shells, meats and meat slices. Wild red abalone are larger in multiple measures of size distinguishing them from legally sold farmed red abalone. We find that abalone meat weights and lengths are correlated to shell length so that the original shell length of the abalone can be estimated even when the shells are not available to enforcement officers for prosecution.

INTRODUCTION

Since the statewide closure of the commercial fishery in 1997 (SB 463), it has been illegal to buy and sell abalone taken from the wild in California (Cal. Fish & Game Code Sec. 7121). Nevertheless, thousands of red abalone, *Haliotis rufescens*, taken in the recreational fishery north of San Francisco are unlawfully sold every year (Daniels and Floren 1998). This illicit commercial trade threatens the sustainability of northern California's red abalone stocks. In response to this threat, State Senator Wes Chesbro (D) is pursuing legislation that would make the illegal commercialization of wild abalone a felony. Because farmed abalone may be legally sold, a forensic method of differentiating between wild and farmed abalone is needed.

Wild red abalone are taken in the recreational fishery north of San Francisco, which is the only abalone fishery open in the state. Abalone populations in California once supported a commercial fishery landing in excess of 2,000 mt per year, but stocks declined due to overfishing, sea otter predation and disease. In southern California, some populations are <1% of their original abundances (Rogers-Bennett et al. 2002). In contrast, the wild fishery on the north coast has been sustainable with roughly 35,000 divers and pickers who fish an estimated 265,000 abalone per year in 2002 (Kalvass and

Geibel 2006). Gear restrictions prohibiting SCUBA, season closures, yearly and daily bag limits as well as size limits are used to manage this fishery.

The minimum legal size in the recreational fishery is 178 mm (7 inches), while the largest red abalone available commercially from aquaculture farms are less than 160 mm (<6.3 inches) in shell length. This difference in size is sufficient to allow enforcement officers to easily recognize the larger, recreationally caught wild abalone when they are illegally possessed for sale. If an abalone is larger than 178 mm and the shell is attached, officers can use the shell measurement to prosecute by ruling out the possibility that it was obtained lawfully from an aquaculture facility. However, if the shell is removed it is currently impossible for officers to establish its original size and thereby its illicit origin. Absent additional evidence, the only way to demonstrate that an abalone came from the wild is by its shell size. A way to back-calculate the size of an abalone shell from the meat tissue alone is needed but, to date, has not been developed.

Here we examine differences in the size of meats and meat slices from legal size wild and farmed red abalone. To do this, we have taken multiple measures of abalone size from shells, meats and meat slices from two groups: legal size wild red abalone and the largest commercially available farmed abalone. We present methods for back-calculating shell length from whole animals, meat weights, and foot area. We determined multiple measures of size for slices of abalone meat from wild and farmed red abalone. We discuss how these morphometric tools can be used by enforcement to combat the illegal commercialization of red abalone in northern California.

METHODS

Legal size red abalone (N=50) were collected from Van Damme State Park, Mendocino County in northern California (Fig. 1) in February 2002. Red abalone ranged in size from 177 to 213 mm, averaging 197.3 mm. Farmed red abalone were obtained from the Monterey Abalone Company in February 2003. Farmed red abalone (N=50) ranged in size from 148 to 160 mm, averaging 153.7mm. These were the largest farmed red abalone available for purchase in the state.

Whole abalone were measured and weighed. The length of the shell was measured along the longest axis. Abalone were removed from the shell (shucked) and the weight of the shell and whole abalone were taken separately: animal weight. The weight of the abalone foot muscle without the viscera was recorded as meat weight. Once cleaned, the abalone meat is made up of the foot which is approximately oval in shape and the muscle attachment, which is above the foot and is also oval in shape. Slices of meat which included this muscle attachment were considered center cuts. The dimensions of the meat were measured including length and width of the muscle attachment, and the height of the muscle attachment as well as the length and width of the base of the meat, and the height of the total meat. We assumed that the shape of the bottom of an abalone foot is an ellipse, so that the area was estimated using the formula for the area of an ellipse = radius of the long dimension x radius of the short dimension x π.

The abalone meat was sliced and dimensions of the slices were taken. Slices were taken from the anterior head region to the posterior back region. All slices included a

Fig. 1. Map of California showing Van Damme State Park in northern California and San Miguel Island in southern California.

portion of the center muscle attachment portion of the abalone. Edge slices were not used in the analysis. The length and height of each center cut slice of abalone from both the wild and farmed abalone were taken.

RESULTS

Whole red abalone from the wild were larger in length and weight compared with the farmed abalone. Wild abalone averaged 197 mm in shell length and 1640 g in wet weight (Fig. 2). Farmed red abalone were smaller averaging 153.7 mm shell length and 620 g in wet weight. Whole animal wet weights minus the shell were also much greater for the wild (1129 g) compared with the farmed (268 g) red abalone, as were meat weights which were 610 g for the wild and 240 g for the farmed.

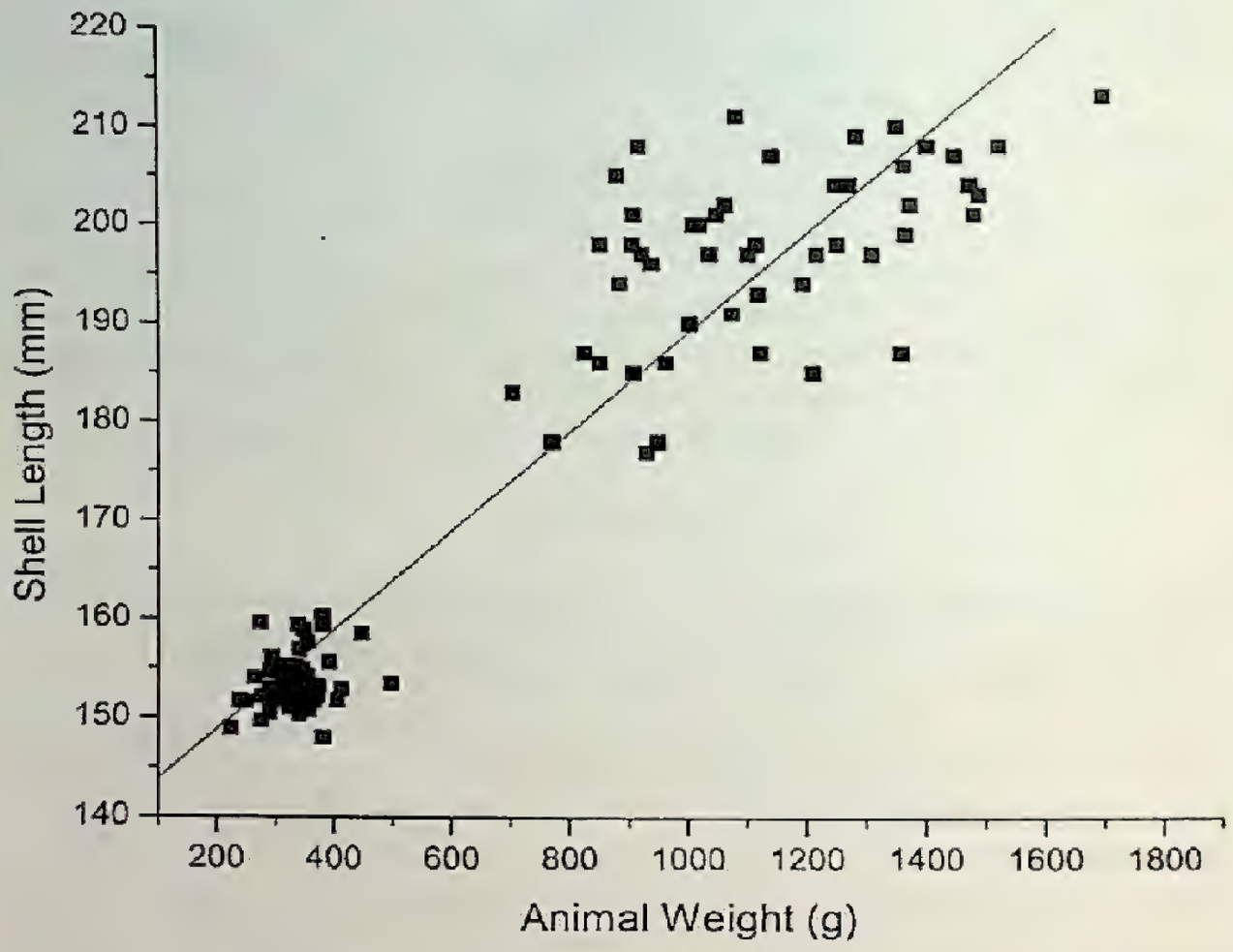

Fig. 2. Linear regression of the weight (g) of a red abalone without the shell plotted against the length of the shell (mm).

Animal weight was highly correlated ($P<0.0001$) with shell length (R=0.945, SD=7.5, N=100). This correlation makes it possible to back calculate to shell size using animal weight. Assuming that the whole intact abalone without the shell can be weighed, this can be used to estimate the length of the shell the abalone came from. Animal weights more than 781 g are estimated to be from abalone more than 178 mm in shell length using the formula:

$Y = 0.05021X + 138.75$
where
X = Animal weight (g)
Y = Shell length (mm)

Meat weight was also highly correlated ($P<0.0001$) with shell length (R=0.872, SD=11.3, N=100). This correlation makes it possible to back-calculate shell length using the whole meat weight without the shell or the viscera (guts). Meats weights more than 453 g are estimated to be from abalone more than 178 mm in shell length (Fig. 3). This method can be used as long as there is at least ½ of the meat available for weighing. The weight of the anterior (with the head) of the abalone was not significantly different than the weight of the posterior half of the abalone (N=28) and so the meat of half the abalone could be multiplied by 2 to get the weight of the whole abalone meat. The formula converting meat weight to shell length is:

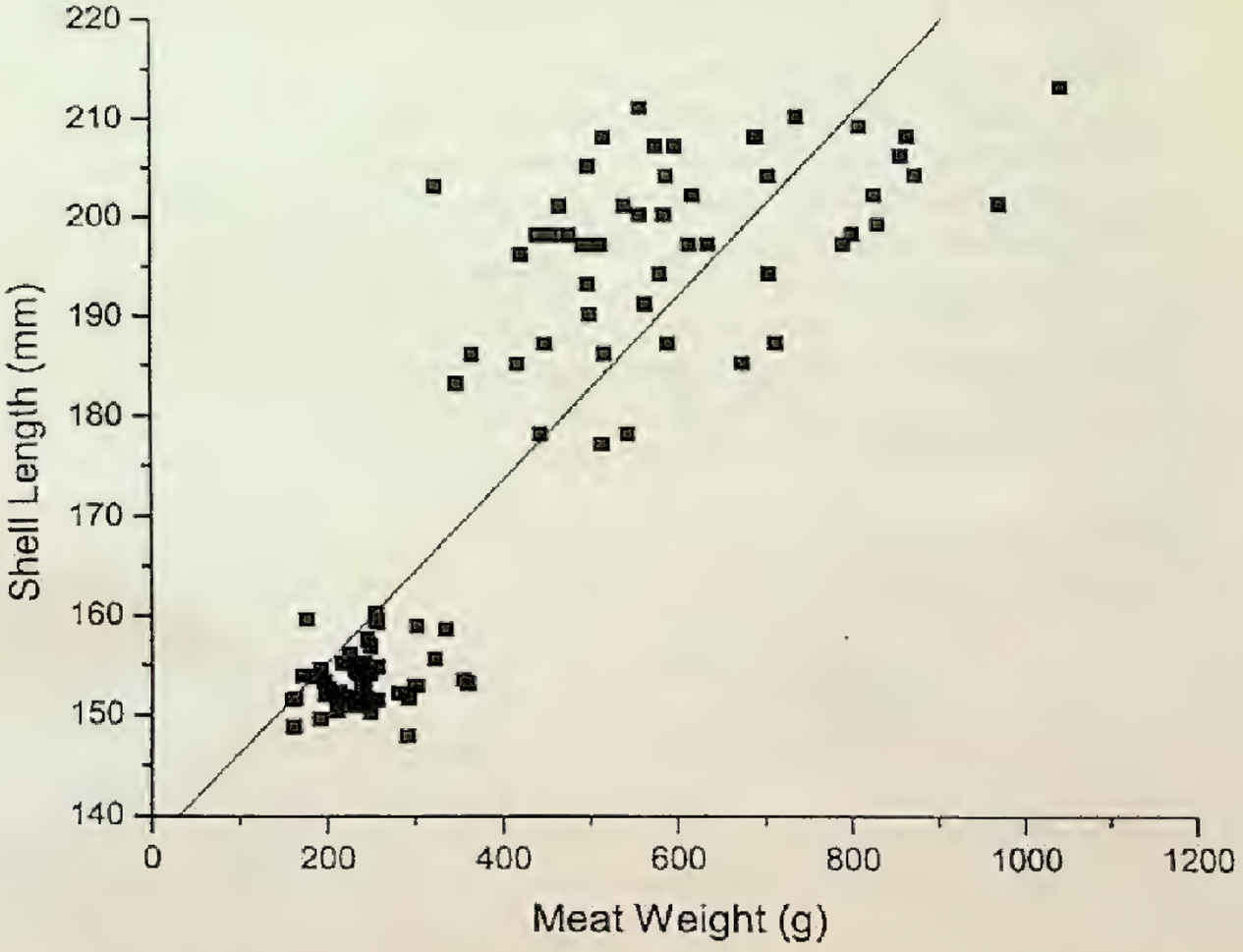

Fig. 3. Linear regression of the weight (g) of a red abalone without the shell and without the viscera plotted against the length of the shell (mm).

$Y = 0.08982X + 137.27$
where
X = Meat weight (g)
Y = Shell length (mm)

Foot area was also highly correlated ($P<0.0001$) with shell length (R=0.914, SD=9.32, N=100). This correlation makes it possible to back-calculate shell length using length and width measures of the foot, assuming you have more than ½ of the foot remaining. The two measures that are needed are the radius of the long dimension and the radius of the short dimension. Foot areas more than 13,184 mm² are estimated to be from abalone more than 178 mm in shell length (Fig. 4).

$Y = 0.00357X + 130.93$
where
X = Foot area (mm²)
Y = Shell length (mm)

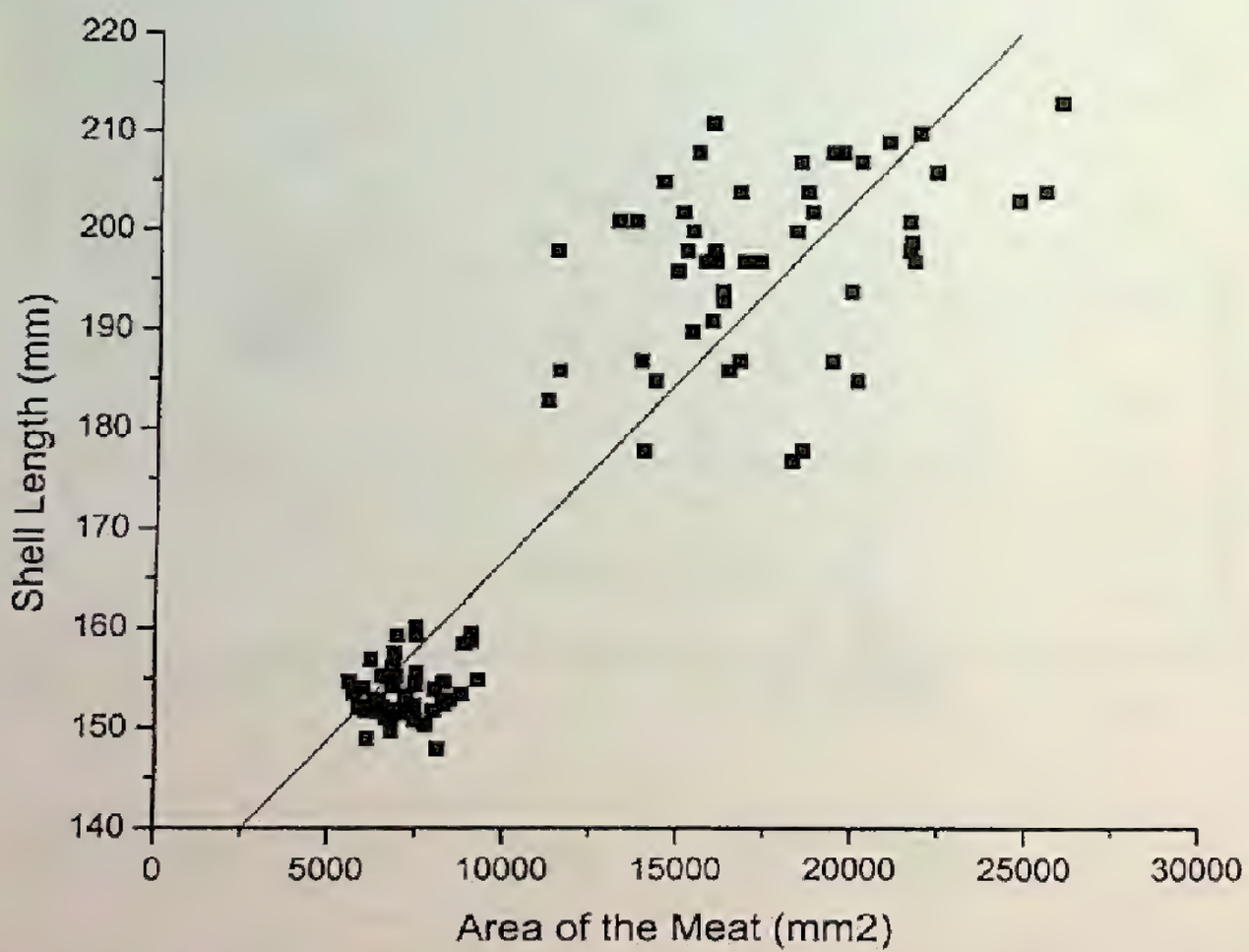

Fig. 4. Linear regression of the area of the foot of the elliptically shaped red abalone (mm²) plotted against the length of the shell (mm).

Slices of abalone meat differed in size between the wild and farmed groups. There was no overlap between groups in the length of the abalone slices taken from the center cut of the abalone meat. Abalone meat slices <80 mm in slice length were all farmed abalone while those larger than 83 mm ranging up to 140 mm were slices from wild abalone. Slice lengths from wild abalone averaged 90.75 mm (SD=12.8) while slice lengths from farmed abalone averaged 75.00 mm (SD=7.5).

DISCUSSION

We present morphometry measures of abalone meat sizes that we demonstrate are correlated with the size of the abalone's shell. When enforcement officers find an abalone meat without the shell they will be able to use the regression relationships developed here to back-calculate an estimate of the abalone shell's length. Measures of foot weight, dimensions of foot area and the length of slices of abalone meat can all be used to estimate the original size of the abalone. Morphometric tools such as the ones developed here can be used to aid wildlife enforcement officers in prosecuting poachers illegally selling abalone in California. Furthermore, enforcement would benefit from additional legislation implementing an upper size limit for farmed abalone, explicitly prohibiting the sale of large abalone (shell length >160 mm) to distinguish them from wild abalone.

A novel form of illegal commercialization has developed in California, in which lega size (>178 mm) abalone taken in the recreational fishery are sold (A. Melvin unpublished data). Abalone are sold for personal consumption in and around the San Francisco Bay area, other metropolitan areas and in restaurants. Abalone sold in restaurants and sushi bars are usually prepared by vertically cutting 3-5mm thick slices which are served raw. Legal size abalone in the recreational fishery (>178 mm) yield slices that are larger than farmed abalone legally sold, which we show are smaller in length. This difference in size can be used to distinguish illegally sold slices of wild abalone from legally sold farmed abalone sold as sushi. Since restaurant buyers prefer larger abalone, which are widely available, it is unlikely that small wild abalone (shorts) less than 178 mm would be sold (A. Melvin personnel observation).

Fish businesses are required to maintain records of legally purchased farmed abalone for three years (F&G Code Sec 8050). Fish commercialization is pervasively regulated in California and enforcement officers may inspect all parts of the business without a warrant or probable cause (F&G Code Sec. 7702, F&G Code Sec. 1006). These laws would seem adequate to facilitate the prosecution of restaurateurs illegally selling wild caught abalone, and yet they are not. Often, prosecutors can only establish that restaurateurs did not have proper documentation. With no empirical method of distinguishing wild and farmed abalone, they cannot prosecute for illegally selling wild abalone. Failure to keep fish business records is punishable by a maximum fine of $1000 with no minimum fine. However, the unlawful sale of abalone carries a maximum fine of $40,000 (minimum $15,000) (F&G Code Sec. 12002.3(b)). Clearly, when restaurateurs are fined or reprimanded for not having farmed abalone receipts they are not receiving a punishment commensurate with the unlawful purchase/sale of abalone. Therefore, the

formulas developed here can be used by enforcement officers at the point of sale to detect the unlawful sale of abalone rather than the lesser crime of failure to maintain purchase records.

Currently (2006), there is a proposal before the California Fish and Game Commission to re-open a commercial red abalone fishery in southern California by 2008 (Fig. 1) around San Miguel Island (P. Coulston personnel communications). At this point, it is unknown what regulations might be implemented for this potential fishery, but any decision regarding size limits will directly effect the enforcement of laws prohibiting the sale of recreationally caught abalone in California and the application of the methods developed here to combat illegal commercialization.

Abalone poaching in California and elsewhere around the world is extremely lucrative and is infamous for negatively impacting the sustainability of abalone fisheries worldwide (Hauck and Sweijd 1999, Jamieson 2001, Huchette and Clavier 2004). In South Africa, abalone poachers have armed confrontations with enforcement officers and estimates of the illegal take suggest that it may be as great, or greater than the legal take, threatening to cause the collapse of the fishery (Tarr 2000). In northern California, effective enforcement will be critical to maintaining healthy abalone stocks in one of the last open abalone fisheries on the west coast of North America.

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LIFE HISTORY AND ECOLOGICAL CHARACTERISTICS OF THE SANTA ANA SUCKER, CATOSTOMUS SANTAANAE

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This study was conducted to document the life history and ecological characteristics of the Santa Ana sucker, *Catostomus santaanae*, within its native range in southern California. Electrofishing surveys were conducted at 3-month intervals from December 1998 to December 1999 at one site on the San Gabriel River and two sites on the Santa Ana River. Suckers were captured in the San Gabriel River (average, 6.6 fish/10-minutes electrofishing) and at an upstream Santa Ana River site (average, 2.3 fish/10-minutes electrofishing) but not at a downstream Santa Ana River site. Length frequency distributions indicated that at least three year classes (modal groups) of suckers were present in the San Gabriel River, whereas one or two year classes were present in the Santa Ana River. Collection of 21-30 mm standard length (SL) juveniles in June in the Santa Ana River and in September in the San Gabriel River indicated that reproduction occurred over several months. In December, Age-0 suckers averaged 36-48 mm SL in the San Gabriel River and 63-65 mm SL in the Santa Ana River, whereas Age-1 suckers averaged 86 mm SL in the San Gabriel River and 115 mm SL in the Santa Ana River. On average, suckers were in better body condition in the San Gabriel River than in the Santa Ana River. Highest abundance of suckers was associated with relatively pristine environmental conditions (especially low specific conductance) where other native fishes were also common or abundant.

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INTRODUCTION

The Santa Ana sucker, *Catostomus santaanae*, is endemic to the Los Angeles, San Gabriel, and Santa Ana river drainages of southern California (Smith 1966; USFWS[3] 2000). Although commonly found in these drainages during the 1970s, Moyle and Yoshiyama[4] (1992) believed that only the San Gabriel River population was viable and self-sustaining over multiple generations. Recent studies have documented the species in portions of the Santa Ana River and Big Tujunga Creek, a tributary of the Los Angeles River (USFWS[3] 2000). Although Smith (1966) and Swift et al. (1993) reported that suckers in the Santa Clara River represented an introduced population that had hybridized with the Owens sucker, *C. fumeiventris* (also introduced), Buth and Crabtree (1982) found no genetic evidence of introgression among 200 morphologically "pure" Santa Ana suckers they examined.

The USFWS[3] (2000) estimated that habitat loss and degradation had eliminated the Santa Ana sucker from 75% of its native range. In response to evidence of declining populations, the sucker was listed as a threatened species under the Endangered Species Act (USFWS[3] 2000).

Although Greenfield et al. (1970) and Buth and Crabtree (1982) described selected aspects of the life history and ecology of Santa Ana suckers in the Santa Clara River, few published studies exist for this species in other waters. The purpose of our study was to develop a better understanding of the life history and ecological characteristics of this sucker within the San Gabriel and Santa Ana rivers. Specific objectives were to 1) determine the abundance of suckers and co-occurring fishes at selected sites along the San Gabriel and Santa Ana rivers, 2) determine if length-frequency distributions and body condition indices of suckers differed among the sites, 3) document the general habitat features (including water quality) of the sites, and 4) identify physicochemical and biological variables most strongly associated with abundance of suckers.

STUDY AREA AND METHODS

The study area consisted of three sampling sites within the Los Angles Basin (Fig. 1). The first sampling site, hereafter referred to as "SGR," was located on the East Fork of the San Gabriel River (34°14'22"N, 117°45'54"W; elevation, 580 m). The second sampling site, hereafter referred to as "MWD," was located on the Santa Ana River near the Metropolitan Water District's pipeline crossing (33°58'06"N, 117°26'47"W; elevation, 209 m). The third sampling site, hereafter referred to as "IMP," was located on the Santa Ana River near the Imperial Highway bridge (33°51'29"N, 117°47'19"W; elevation, 86

[3]USFWS (U.S. Fish and Wildlife Service). 2000. Endangered and threatened wildlife and plants; threatened status for the Santa Ana sucker (50 CFR Part 17, RIN 1018-AF34). Federal Register 65(71):19686-19698.

[4]Moyle, P.B. and R.M. Yoshiyama. 1992. Fishes, aquatic diversity management areas, and endangered species: a plan to protect California's native aquatic biota. California Policy Seminar, University of California, Berkeley, California, USA.

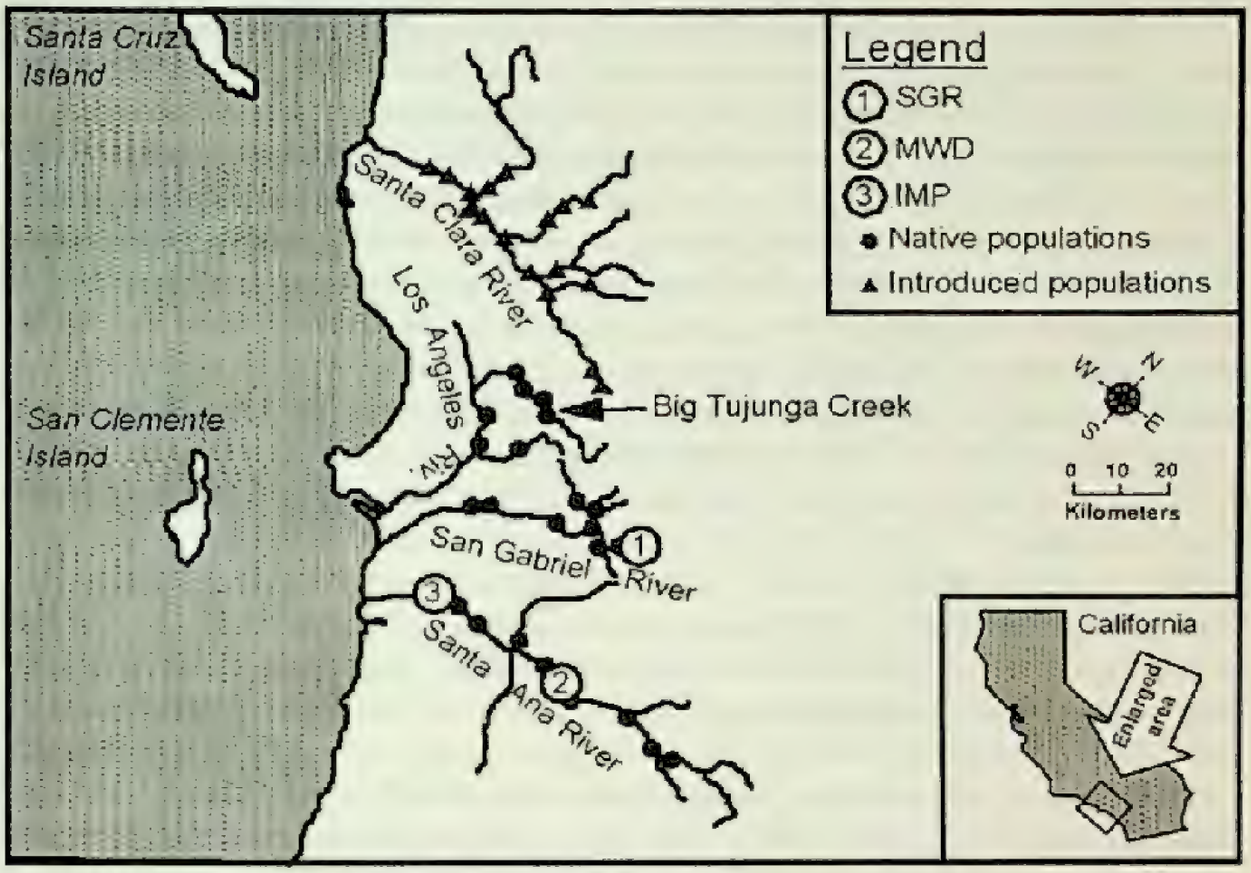

Figure 1. Map of study area showing locations of sampling sites on the East Fork of the San Gabriel River (SGR), the Santa Ana River at the Metropolitan Water District Pipeline crossing (MWD), and the Santa Ana River at Imperial Highway (IMP). Also shown is the documented distribution of native and introduced populations of Santa Ana suckers (map redrawn from Swift et al. 1993).

m). Each sampling site consisted of an upstream reach and a downstream reach (each about 0.4 km in length) separated by a linear distance of about 0.2 km. The sites were chosen to encompass different population densities of the Santa Ana sucker based on unpublished reports (e.g., Chadwick and Associates[5] 1992; Swift[6] 2001) and published agency reports (Deinstadt et al.[7] 1990; USFWS[3] 2000) indicating that this species was abundant at SGR, common at MWD, and rare or absent at IMP.

[5]Chadwick and Associates. 1992. Santa Ana River use-attainability analysis, Volume 2: aquatic biology, habitat and toxicity analysis, report prepared for the Santa Ana Watershed Project, Riverside, California, USA.

[6]Swift, C.C. 2001. The Santa Ana sucker in the Santa Ana River: distribution, relative abundance, spawning areas, and impact of exotic predators. Final report submitted by Larry Munsey International to the Santa Ana Water Project Authority, Riverside, California, USA.

[7]Deinstadt, J.M., E.J. Pert, F.G. Hoover, and S. Sasaki. 1990. Survey of fish populations in six southern California streams: 1987. California Department of Fish and Game, Inland Fisheries Administrative Report No. 90-1, Sacramento, California, USA.

At SGR, the habitat consisted of numerous coarse gravel-cobble bottomed pools, riffles, and glides in a narrow steep-sided canyon containing little riparian vegetation. Stream flow at this site originated entirely from natural runoff. At MWD, the habitat consisted mainly of shallow glides flowing over a shifting-sand substrate in a relatively broad river channel lined by riparian vegetation (giant reed, *Arundo donax*; cattails, *Typha* spp.; and willow, *Salix* sp.). During summer months, the main source of water at this site was discharge from the Rapid Infiltration and Extraction (RIX) Plant, an experimental tertiary wastewater treatment facility in Colton, California. At IMP, the habitat consisted of relatively deep sandy or gravelly bottomed glides with sparse riparian vegetation. During summer and autumn 1999, construction activities in the upper portion of this site funneled stream flow into a relatively narrow artificial channel that fed into a percolation pond (the percolation pond was designed to recharge the groundwater).

Fish collection, using a battery-operated backpack electroshocker (Smith-Root Model 12-B electrofisher) at the lowest output voltage setting needed to stun fish, was initiated at all sampling sites in December 1998 with subsequent collections occurring at roughly 3-month intervals through December 1999. Each of two reaches within a site was electrofished for 50 to 80 minutes, starting downstream and working upstream with one pass completed per reach. Each pass was divided into 5 to 8 sections, based on 10 minutes of shocking time as determined by a voltage-activated timer on the variable voltage pulsator unit. Stunned fish were retrieved with dip nets (3.2-mm mesh) and placed into plastic buckets filled with site water. Immediately after completing a section of the pass, Santa Ana suckers were removed from the catch, measured for standard length (SL; ±1 mm) and weight (±0.1 g), then returned to the water at least 20-m downstream from yet-to-be fished upstream areas. All other fish were identified, counted, and released in the same downstream vicinities.

Greenfield et al. (1970) found that scale analysis yielded unreliable estimates of Santa Ana sucker ages. Moreover, its status as a federally protected species limited sacrifice of suckers for examination of otoliths or other bony structures. Due to these reasons, we used length-frequency histograms to estimate age classes of sucker populations.

Length-weight relations for Santa Ana suckers from each site and quarterly collection were computed from the formula, $\log_{10} W = a + b * \log_{10} L$, where "a" is the intercept, "b" is the slope, W is weight, and L is standard length (Bagenal and Tesch 1978). A generalized length-weight equation was constructed from unweighted quarterly means of intercepts and slopes for all sites and used to compute the relative condition factor (K_n) of each sucker (Bagenal and Tesch 1978). Relative condition factor was computed from $K_n = W/W'$ where W is the actual weight and W' is estimated from the generalized length-weight equation.

Water temperature, dissolved oxygen, pH, specific conductance, and turbidity were measured within the water column with Hydrolab DataSonde 4 multiprobe loggers at 15-minute intervals from a fixed station located at the upstream boundary of each sampling reach. Measurements were taken only during daylight hours when field personnel were at the sampling site (about 8-10 hours). Channel width was measured

at each fixed station per reach, and current velocity (determined with a Price Type "AA" model 1210 current meter) and water depth were measured at five equidistant points along the cross-sectional profile of the channel. Stream discharge for each reach was estimated from these data. In addition, we characterized microhabitat conditions for Santa Ana suckers by measuring water temperature, dissolved oxygen, pH, specific conductance, turbidity, water velocity, depth, habitat type (pool, riffle, or glide), predominant substrate (silt, sand, gravel, cobble, or boulder), and predominant cover (submerged cobble, boulders, or manmade structures; submerged macrophytes or filamentous algae; and emergent aquatic macrophytes or riparian vegetation) wherever individual suckers were captured within the various sampling reaches. Determinations of habitat type, predominant substrate, and predominant cover were subjectively made by visual inspection.

With one exception, summary statistics and tests of statistical significance were computed with SAS software (SAS Institute Inc. 1990). Unless indicated otherwise, all computations were made with data transformed to $\log_{10}$. Variations in fish species composition among sites were assessed by using the chi-square (χ^2) test for homogeneity with counts (numbers) of fish captured during all quarterly collections. Length-weight relationships for Santa Ana suckers captured from each site and quarterly collection were determined by simple linear regression. A one-way analysis of variance (ANOVA) was used to compare mean K_n values of suckers from SGR and MWD for each quarterly sample. MIX software (Macdonald and Green[8] 1988) was used to analyze length-frequency distributions. Pearson's product-moment correlation was used to detect associations between sucker abundance and selected physicochemical and biological variables. Unless specified otherwise, the level of significance for all statistical tests was $P = 0.05$.

RESULTS

A total of 7,307 fish represented by 16 species was captured during the five sampling trips (Table 1). The proportions of various fish species differed significantly among the three sites ($\chi^2 = 12{,}799$, $df = 30$, $P < 0.0001$). Overall, SGR supported the fewest fish species, with native fishes (Santa Ana sucker; speckled dace, *Rhinichthys osculus*; arroyo chub, *Gila orcutti*; and rainbow trout, *Oncorhynchus mykiss*) comprising 100% of the catch. By comparison, MWD supported only two native species (suckers and arroyo chub; 47% of catch) and eight nonnative species. Ten species were captured at IMP, all of which were nonnative.

On average, the abundance (number of individuals/10-minutes electrofishing) of all fishes and native fishes was highest at SGR (40.7 and 40.7 fish/10-minutes electrofishing), followed by MWD (20.0 and 9.4 fish/10-minutes electrofishing), then by IMP (12.5 and 0.0 fish/10-minutes electrofishing). The abundance of nonnative

[8]Macdonald, P.D.M. and P.E.J. Green. 1988. User's guide to program MIX: an interactive program for fitting mixtures of distributions. Ichthus Data Systems, Hamilton, Ontario, Canada.

Table 1. Abundance (number of individuals/10-minutes electrofishing) and percent (%) of fish species from the East Fork of the San Gabriel River (SGR), the Santa Ana River at the Metropolitan Water District Pipeline Crossing (MWD), and the Santa Ana River at Imperial Highway (IMP). Values for abundance are unweighted geometric grand means computed from five seasonal geometric means.

Family	Species	Status	SGR		MWD		IMP	
			Abundance	%	Abundance	%	Abundance	%
Catostomidae	Santa Ana sucker, *Catostomus santaanae*	Native	6.58	16.14	2.32	11.62	0.00	0.00
Centrarchidae	Green sunfish, *Lepomis cyanellus*	Nonnative	0.00	0.00	0.09	0.44	0.13	1.05
	Bluegill, *Lepomis macrochirus*	Nonnative	0.00	0.00	0.00	0.00	0.27	2.19
	Largemouth bass, *Micropterus salmoides*	Nonnative	0.00	0.00	0.03	0.15	0.71	5.69
Cichlidae	"Tilapia"[a]	Nonnative	0.00	0.00	0.06	0.32	0.00	0.00
Cyprinidae	Goldfish, *Carassius auratus*	Nonnative	0.00	0.00	0.00	0.00	0.30	2.41
	Common carp, *Cyprinus carpio*	Nonnative	0.00	0.00	0.00	0.00	3.06	24.49
	Arroyo chub, *Gila orcutti*	Native	0.14	0.35	7.11	35.55	0.00	0.00
	Fathead minnow, *Pimephales promelas*	Nonnative	0.00	0.00	0.75	3.73	7.01	56.09
	Speckled dace, *Rhinichthys osculus*	Native	18.68	45.85	0.00	0.00	0.00	0.00
Ictaluridae	Black bullhead, *Ameiurus melas*	Nonnative	0.00	0.00	0.02	0.11	0.03	0.25
	Yellow bullhead, *Ameiurus natalis*	Nonnative	0.00	0.00	0.63	3.16	0.18	1.41
	Channel catfish, *Ictalurus punctatus*	Nonnative	0.00	0.00	0.00	0.00	0.25	1.97
Poeciliidae	Western mosquitofish, *Gambusia affinis*	Nonnative	0.00	0.00	8.95	44.74	0.56	4.46
	Sailfin molly, *Poecilia latipinna*	Nonnative	0.00	0.00	0.04	0.19	0.00	0.00
Salmonidae	Rainbow trout, *Oncorhynchus mykiss*	Native	15.34	37.65	0.00	0.00	0.00	0.00

[a]Although one of five "tilapia" caught during this study was tentatively identified as a redbelly tilapia, *Tilapia zilli* (judging from a reddish-colored throat and belly, and dark vertical bands on the sides), the remainder possibly consisted of juvenile Mozambique tilapia, *Oreochromis mossambicus*, a species known to occur in the Santa Ana River (Swift et al. 1993; Brown et al. 2005).

fishes was similar at MWD (10.6 fish/10-minutes electrofishing) and IMP (12.5 fish/10-minutes electrofishing). Santa Ana suckers were most plentiful at SGR (average, 6.6 fish/10-minutes electrofishing), followed by MWD (average, 2.3 fish/10-minutes electrofishing), and not captured at IMP.

Length-Frequency Distributions of Suckers

A total of 715 length measurements was obtained from Santa Ana suckers captured at SGR and MWD during the five sampling trips. The largest sucker from SGR measured 168 mm SL (89.0 g), whereas the largest sucker from MWD measured 151 mm SL (62.0 g). Judging from quarterly length-frequency distributions of suckers, at least two or three year classes (modal groups) were present at SGR whereas one or two year classes were present at MWD (Fig. 2). In December 1998, the smallest group (1998 cohort) of suckers averaged 36 mm SL at SGR and 63 mm SL at MWD. One year later, the 1998 cohort at SGR averaged 86 mm SL, whereas the same cohort at MWD averaged 115 mm SL. Age-0 suckers were found in June 1999 at MWD and in September 1999 at SGR. By December 1999, Age-0 suckers averaged 48 mm SL at SGR and 65 mm SL at MWD.

Length-Weight Relationship and Relative Condition of Suckers

On several occasions, estimates of slopes, intercepts, or both, from length-weight relationships computed for quarterly samples of Santa Ana suckers differed significantly between collections from SGR and MWD. The overall length-weight relationship was described by $\log_{10} W = -4.265627 + 2.774215 * \log_{10} L$. Mean K_n of suckers varied from 0.99 (March 1999) to 1.13 (Dec 1998) at SGR and from 0.87 (June 1999) to 1.02 (Dec 1999) at MWD (Table 2). Although seasonal geometric means were typically higher in suckers from SGR than from MWD, only three of five comparisons (December 1998, June 1999, and September 1999) were significantly different.

Water Quality and Other Environmental Conditions

Water quality and other environmental conditions varied among the three sampling sites (Table 3). Although seasonal variations exceeded spatial variations, water temperatures were generally cooler at SGR than at MWD and IMP, with MWD typically exhibiting the warmest temperatures. On average, specific conductance, turbidity, stream width, and discharge were lowest at SGR and higher at MWD and IMP, whereas pH was highest at SGR and lower at MWD and IMP. Water depth was shallowest at MWD, intermediate at SGR, and deepest at IMP. Current velocity was highest at IMP and lower at SGR and MWD.

Relation Between Sucker Abundance, Selected Environmental Variables, and Abundance of Other Fish Species

According to Pearson product-moment correlation analysis, the relative abundance of Santa Ana suckers was inversely associated with specific conductance, current

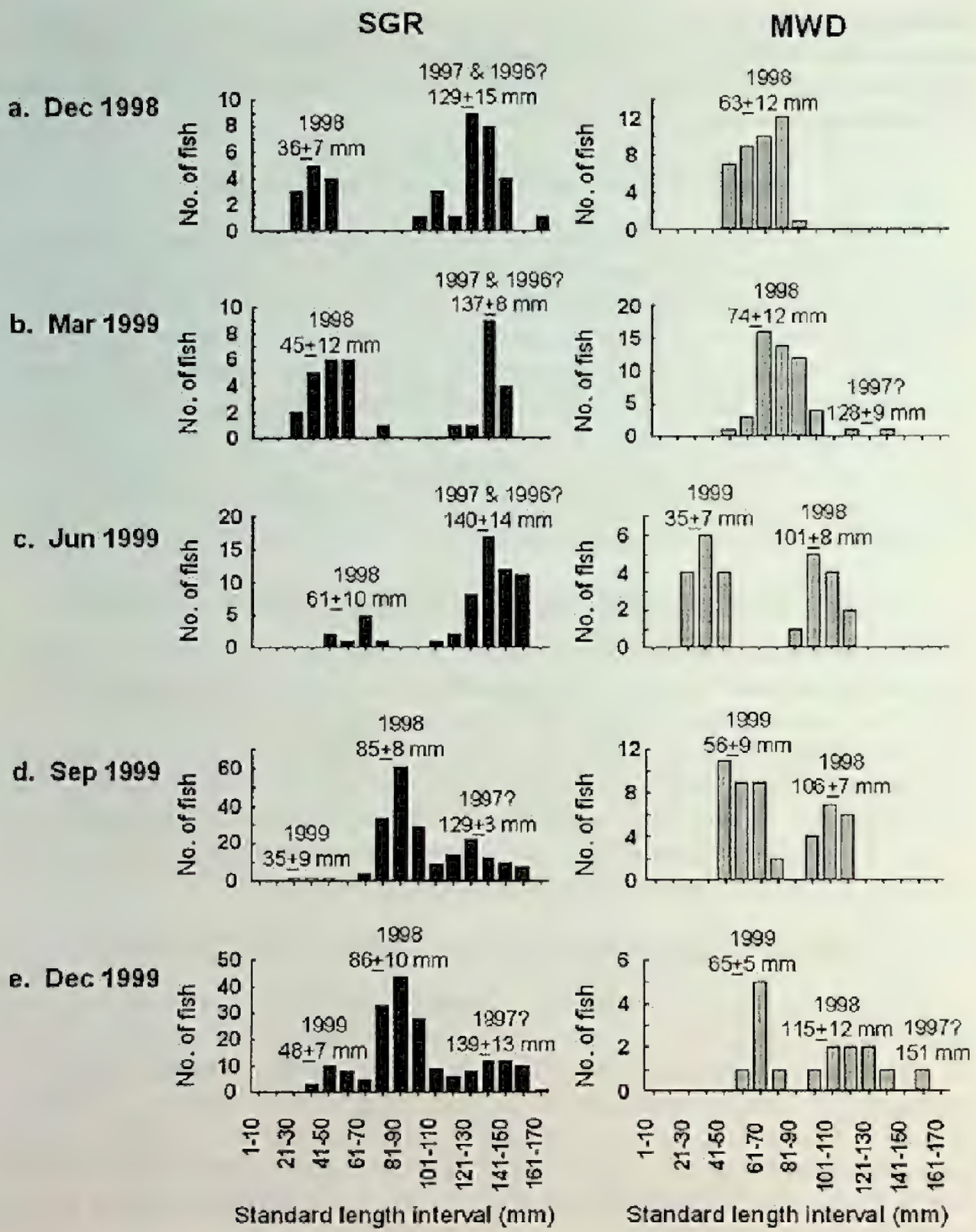

Figure 2. Length frequencies of Santa Ana suckers from the East Fork of the San Gabriel River (SGR) and the Santa Ana River at the Metropolitan Water District Pipeline crossing (MWD), December 1998 to December 1999. Also shown are estimated year class and mean standard length ± SD.

Table 2. Comparison of relative condition factor (K_n) for Santa Ana suckers from the East Fork of the San Gabriel River (SGR) and the Santa Ana River at the Metropolitan Water District Pipeline Crossing (MWD) on five occasions between December 1998 and December 1999. Values for K_n are geometric means (range in parentheses). F statistics are from one-way ANOVA.[a]

Date	Site	N	K_n or F statistic
December 1998	SGR	39	1.13 (0.77-3.42)
	MWD	39	0.92 (0.79-1.17)
			$F_{1,77} = 27.24$***
March 1999	SGR	38	0.99 (0.40-1.50)
	MWD	51	0.95 (0.70-1.40)
			$F_{1,88} = 0.76$
June 1999	SGR	58	1.03 (0.79-2.17)
	MWD	26	0.87 (0.56-1.39)
			$F_{1,83} = 17.86$***
September 1999	SGR	206	1.03 (0.72-2.08)
	MWD	48	0.97 (0.64-2.19)
			$F_{1,253} = 6.16$*
December 1999	SGR	188	1.03 (0.50-1.97)
	MWD	16	1.02 (0.84-1.29)
			$F_{1,203} = 0.10$

[a]Codes: * = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$.

velocity, and stream discharge, and directly associated with abundances of speckled dace and rainbow trout (Table 4). Although not statistically significant ($0.05 > P > 0.002$ to accommodate 24 simultaneous comparisons), weak correlations also existed between sucker abundance and pH, turbidity, stream width, and abundances of largemouth bass, *Micropterus salmoides*, common carp, *Cyprinus carpio*, and fathead minnow, *Pimephales promelas*.

Microhabitat Conditions at Sucker Capture Localities

Although Santa Ana suckers were caught throughout the sites sampled at SGR and MWD, a comparison of microhabitat conditions associated with capture locations of individual fish yielded several major differences. At SGR, suckers were caught mostly

Table 3. Comparison of water quality and other environmental variables from the East Fork of the San Gabriel River (SGR), the Santa Ana River at the Metropolitan Water District Pipeline Crossing (MWD), and the Santa Ana River at Imperial Highway (IMP). Values for temperature, dissolved oxygen, pH, specific conductance, and turbidity are unweighted geometric grand means (95% CI in parentheses) from four site visits made in 1999[a], whereas other variables are geometric grand means (95% CI in parentheses) from five site visits made in 1998-1999.

Variable	SGR	MWD	IMP
Temperature (°C)	12.9 (8.0-21.0)	19.3 (14.4-25.9)	17.0 (12.3-23.5)
Dissolved oxygen (mg/liter)	9.3 (7.9-10.9)	8.0 (6.9-9.3)	9.0 (7.9-10.2)
pH	8.37 (8.32-8.41)	8.12 (8.09-8.15)	8.16 (8.05-8.26)
Specific conductance (µmhos/cm @ 25°C)	350 (310-390)	770 (650-910)	990 (910-1070)
Turbidity (NTUs)	5.9 (4.3-8.2)	29.0 (10.1-83.4)	68.6 (33.0-142.6)
Stream width (m)	9.6 (8.3-11.0)	38.3 (33.8-43.4)	34.4 (23.5-50.4)
Water depth (m)	0.28 (0.25-0.31)	0.17 (0.14-0.20)	0.43 (0.34-0.53)
Current velocity (m/second)	0.24 (0.12-0.47)	0.46 (0.44-0.49)	0.60 (0.53-0.67)
Discharge (m^3/second)	0.63 (0.34-1.17)	2.94 (2.55-3.39)	8.75 (7.60-10.06)

[a]Water quality data for December 1998 were omitted because the pH probe was defective.

in glides (46%), followed by riffles (38%), then pools (16%). By comparison, suckers at MWD were caught almost exclusively in glides (99%), with only two individuals (1%) captured in pools. In addition, suckers were generally caught in deeper and slower moving waters at SGR than at MWD. For example, 532 suckers at SGR were caught in water averaging 0.37 m deep (95% confidence interval, 0.36-0.39 m), whereas 157 suckers at MWD were caught in water averaging 0.22 m deep (95% confidence interval, 0.21-0.23 m). Moreover, suckers at SGR were caught at an average water velocity of 0.29 m/second (95% confidence interval, 0.27-0.31 m/sec), whereas suckers at MWD were caught at an average water velocity of 0.41 m/sec (95% confidence interval, 0.39-0.43 m/sec).

The bottom substrate and predominant cover associated with captured Santa Ana suckers also differed between SGR and MWD. At SGR, nearly 76% of suckers were caught over gravel or cobble substrates, with only about 20% caught over sand. However, at MWD, over 99% of suckers were caught over sand. In addition, at SGR, over 98% of suckers were caught among or adjacent to large cobble, boulders, or artificial structures such as fractured concrete and metal culverts. By comparison, at MWD, less than 1% of suckers were caught within or near such structures, with over 82% caught in stands of emergent aquatic macrophytes (e.g., cattails, bulrush) and 17% caught within submerged riparian vegetation (e.g., roots, branches, overhanging grasses).

Table 4. Pearson's product-moment correlations between abundance (number of fish/10-minutes electrofishing) of Santa Ana suckers and selected physicochemical and biological variables. N = 15 for all variables except pH, where N = 14.

Variable	R	P[a]
Physicochemical:		
Water temperature (°C)	-0.20	0.4716
Dissolved oxygen (mg/liter)	-0.01	0.9786
pH	0.58	0.0293
Specific conductance (µmhos/cm @ 25°C)	-0.79	0.0005*
Turbidity (NTUs)	-0.67	0.0065
Stream width (m)	-0.61	0.0154
Water depth (m)	-0.47	0.0743
Current velocity (m/second)	-0.80	0.0003*
Discharge (m^3/second)	-0.88	<0.0001*
Biological[b]:		
Green sunfish	-0.29	0.3001
Bluegill	-0.50	0.0550
Largemouth bass	-0.72	0.0027
"Tilapia"	0.05	0.8677
Goldfish	-0.43	0.1124
Common carp	-0.68	0.0050
Arroyo chub	0.25	0.3689
Fathead minnow	-0.60	0.0185
Speckled dace	0.74	0.0017*
Black bullhead	-0.21	0.4588
Yellow bullhead	-0.08	0.7834
Channel catfish	-0.44	0.1019
Western mosquitofish	-0.08	0.7767
Sailfin molly	-0.11	0.6884
Rainbow trout	0.75	0.0013*

[a]Code: *, statistically significant according to the adjusted Bonferroni P = 0.0021 for 24 simultaneous comparisons.
[b]Biological variables are expressed as abundance (numbers of fish/10-minutes electrofishing).

DISCUSSION

During our study, native species such as Santa Ana suckers, speckled dace, arroyo chub, and rainbow trout occurred in the San Gabriel River, but only Santa Ana suckers and arroyo chub occurred in the Santa Ana River. These findings are consistent with fish surveys conducted over the past two decades by private consultants under contract to local government agencies (Chadwick and Associates[5] 1992; Swift[6] 2001) and the California Department of Fish and Game (Deinstadt et al.[7] 1990). Recently,

Brown et al. (2005) documented the occurrence of native and nonnative fishes, including speckled dace and rainbow trout, from 17 sites in the Santa Ana River system. However, the dace and trout captured by Brown et al. (2005) came from sites located farther upstream and at higher elevations than the sites we sampled in the Santa Ana River.

At least three age classes characterized the Santa Ana sucker population at SGR whereas one or two age classes usually characterized the sucker population at MWD (Fig. 2). Chadwick and Associates[5] (1992) reported two size groups of suckers in their surveys of fishes from the Santa Ana River although they did not assign the size groups to age classes because they lacked data collected over time. By comparison, Greenfield et al. (1970) noted that a few suckers lived as long as 4 years in the Santa Clara River. These ages are less than those reported for the closely related mountain sucker, *Catostomus platyrhynchus*, which reportedly lives for 7 to 9 years (Moyle 2002).

The proportion of large (>100 mm SL) Santa Ana suckers declined at both SGR and MWD between June and September 1999. Similarly, Smith (1966) and Greenfield et al. (1970) reported a decline in abundance of larger specimens from early summer through autumn, but neither Smith (1966) nor Greenfield et al. (1970) offered insights to the cause of this phenomenon. The suckers may have just reached the end of their natural life span.

Age-0 fish attained average lengths of 36–48 mm SL at SGR and 63-65 mm SL at MWD by December, and Age-1 fish averaged 86 mm SL at SGR and 115 mm SL at MWD by December. By comparison, Greenfield et al. (1970) reported that Santa Ana suckers in the Santa Clara River reached 30 to 70 mm SL (average, 44 mm SL) during their first year of life and 77 to 110 mm SL during their second year of life. The relatively small average sizes attained by Age-0 and Age-1 suckers from SGR and the Santa Clara River might be related to the higher elevations of these localities—about 580 m at SGR and 530-620 m at the Santa Clara River compared to 209 m at MWD. Elevations of sites sampled by Greenfield et al. (1970) in the Santa Clara River were estimated from 7.5' topographic maps available at http://maps.nationalgeographic.com/topo/. Presumably, suckers inhabiting high-elevation rivers experience cooler ambient water temperatures that lower their metabolic (growth) rates and reduce the length of their growing seasons.

According to Carlander (1969), a length-weight relationship containing a slope <3.0 indicates that weight decreases relative to length as the fish grows. Our data yielded a slope of 2.77, suggesting that Santa Ana suckers became slimmer (less plump) as they increased in length. On average, suckers also exhibited lower body condition at MWD than at SGR. Although beyond the scope of our study, we suspect that the lower body condition in suckers from MWD resulted from an inadequate food supply, relatively warm water temperatures, and other potentially stressful situations. Moyle (2002) noted that Santa Ana suckers feed mostly on detritus and algae, which they scrape from rocks and other surfaces. In the Santa Clara River, over 97% of the diet of Santa Ana suckers consisted of detritus, algae, and diatoms, with the remainder consisting of aquatic insects (Greenfield et al. 1970). Periphyton and insects were scarce on the shifting sand substrate at MWD but relatively abundant on the rocky substrate at SGR (M.K. Saiki, unpublished observations). Moreover, ambient water temperatures were

generally higher at MWD than at SGR, presumably increasing the metabolic requirements of suckers at MWD. Collectively, these conditions at MWD could have prevented suckers from accumulating large amounts of fat reserves, resulting in their lower body condition. Negative ecological interactions with nonnative fishes, which were rare or absent at SGR but common or abundant at MWD, might have also stressed suckers at MWD and contributed to low body condition.

The first occurrence of juvenile (≤30 mm SL) Santa Ana suckers in our study was documented in June at MWD when daytime water temperatures averaged 25.3°C (range, 17.6-30.8°C) and in September at SGR when daytime water temperatures averaged 19.6°C (range, 15.0-22.9°C), indicating that spawning had occurred in both rivers several weeks or months earlier. Although Greenfield et al. (1970) examined embryolarval development in the Santa Ana sucker by incubating artificially fertilized ova at 13°C in the laboratory, to our knowledge, the temperature range over which reproduction naturally occurs has not been determined. Swift[6] (2001) observed large numbers of sucker larvae upstream from MWD in mid-April 2000, suggesting that spawning had occurred as early as mid-March in the Santa Ana River. By comparison, Greenfield et al. (1970) reported that suckers spawned from early April through early July in the Santa Clara River, with peak spawning occurring in late May through June. Smith (1966) collected sucker larvae from an unspecified location in the Santa Clara River in late November. Collectively, these observations suggest this species can spawn over an extended time period, possibly 6-8 months in duration.

The abundance of Santa Ana suckers was inversely correlated with specific conductance, current velocity, and stream discharge, and directly correlated with abundance of speckled dace and rainbow trout (Table 4). These patterns reflect major differences in environmental conditions and fish species occurring at SGR (high gradient topography with relatively pristine environmental conditions; native species dominant) where suckers were numerous, and MWD and IMP (lower gradient topography with disturbed environmental conditions; nonnative species dominant) where suckers were less numerous or absent.

Santa Ana suckers occupied nearly all habitat types available at both SGR and MWD. Although less than half the suckers at SGR occurred in runs whereas nearly all suckers at MWD occurred in runs, this difference was seemingly a consequence of the availability of various habitat types within the two sites (pools and riffles were a conspicuous part of the landscape at SGR but almost nonexistent at MWD). Similarly, a larger percentage of suckers occurred in deeper water and over coarser bottom substrates at SGR than at MWD because deep water (>0.50 m) and large-grained substrates (gravel, cobble, and boulders) were commonplace at SGR but virtually nonexistent at MWD. Likewise, suckers at SGR were often captured in mid-channel adjacent to submerged cobble, boulders, or manmade structures (culverts) whereas suckers at MWD were usually captured within 1-2 m of the shoreline (and almost never in mid-channel) because riparian vegetation, aquatic macrophytes, and other potential cover occurred almost exclusively onshore or adjacent to shore. These observations agree with Moyle and Yoshiyama[4] (1992), who reported that overhanging riparian plants provide cover for Santa Ana suckers although this species can use the entire

stream and does not require streamside cover when larger, deeper holes and riffles are available.

In conclusion, findings from our study support the contention of many resource managers that the San Gabriel River supports a healthy population of Santa Ana suckers whereas the Santa Ana River supports a marginal population of suckers. Reproduction occurred annually in both river systems, with Age-0 and Age-1 fish attaining larger sizes by December at MWD than at SGR. However, few fish seemingly reached Age 2 at MWD, whereas fish of this age were relatively numerous at SGR. Moreover, fish from MWD were often in lower body condition than fish from SGR, possibly due to inadequate food resources, warmer water temperatures, or the presence of nonnative fishes. Our inability to capture suckers at IMP suggested that they were either very rare or absent downstream in the Santa Ana River. However, in 2000, Baskin and Haglund[9] (2001, cited in USFWS[10] 2004) captured 10 suckers immediately upstream from IMP, although it is not known if the fish were established residents or simply accidental wanderers from more favorable upstream reaches. Environmental variables most strongly associated with high abundance of suckers included low specific conductance, low stream discharge, and high abundances of Santa Ana speckled dace and rainbow trout. In the Los Angeles basin, such conditions are found at moderately high elevations in the San Gabriel River where nonnative species are rare or absent and pollution or other forms of human disturbance are minimal. However, other variables may also influence sucker abundance because Brown et al. (2005) did not find suckers in upper reaches of the Santa Ana River system where dace and trout were present. To protect this species from possible extinction, we concur with Moyle and Yoshiyama[4] (1992) and Moyle (2002) that native fish sanctuaries should be established in much of the San Gabriel River system and perhaps in selected reaches of the Santa Ana and Los Angeles river drainages where the sucker is still extant. Recently, the USFWS[11] (2005) designated portions of the San Gabriel River and Big Tujunga Creek as critical habitat for the sucker after rejecting an earlier attempt (see USFWS[10] 2004) to also include portions of Little Tujunga Creek and the Santa Ana River.

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[9]Baskin, J.N. and T.R. Haglund. 2001. Santa Ana sucker survey/seining in the Santa Ana River. Prepared for U.S. Army Corps of Engineers, Contract No. DACA09-99-0003.

[10]USFWS (U.S. Fish and Wildlife Service). 2004. Endangered and threatened wildlife and plants; final rule to designate critical habitat for the Santa Ana sucker (*Catostomus santaanae*) (50 CFR Part 17, RIN 1018-AT57). Federal Register 69(38):8839-8861.

[11]USFWS (U.S. Fish and Wildlife Service). 2005. Endangered and threatened wildlife and plants; final rule to designate critical habitat for the Santa Ana sucker (*Catostomus santaanae*); final rule (50 CFR Part 17, RIN 1018-AT57). Federal Register 70(2):425-458.

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MARKING OF NOVEL OBJECTS BY KIT FOXES

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Scent marking of novel objects by kit foxes, *Vulpes macrotis*, is not well understood. Generally, kit foxes leave scats (feces) at dens, trail intersections, fence lines, and along unpaved roads (Egoscue 1962, O'Farrell 1987, Ralls and Smith 2004, Smith et al. 2005). In the closely related swift fox, *V. velox*, researchers commonly found scats near cattle guards, fence intersections, culverts, and intersections of roads and trails (Harrison et al. 2004). Kit foxes occasionally leave scats near or on conspicuous objects, including bottles, tires, and skeletal remains (Ralls and Smith 2004) and are known to mark novel objects with undersized scats (O'Farrell 1987).

Scats from the San Joaquin kit fox, *V. m. mutica*, have been found on or near cement objects, sheep carcasses, coyote skulls, cans, fenceposts, pieces of bone, power line poles, and coyote latrines (Ralls and Smith 2004). In another study of scent marking by urban San Joaquin kit foxes, most marks occurred on or near conspicuous or prominent objects, including large rocks, raised soil mounds, asphalt and concrete surfaces, shrubs, posts/poles, trees, buildings, cars, old food items, and tall vegetation. Males were more likely to mark objects than were females (Murdoch 2004[1]).

Egoscue (1962) described the fecal deposition habits of kit foxes in Tooele County, Utah, and found "scats ... along trails, at dens, and occasionally near objects such as bits of bone or other animal remains." However, frequently visited 'sign' stations (old skeletons or natural objects) were not discovered. Egoscue (1962) further described unique behavior of kit foxes marking a rodent live-trap grid: "One fox left small scats on 15 of 50 traps in a single round of the trap-line, and by the third night had urinated on almost every trap." Egoscue (1962) also reported that after the marking efforts of the kit fox, the trapping success at the rodent live-trap grid dropped considerably. Here I describe fecal marking efforts of the kit fox on a live-trap grid set for diurnal rodents near Ridgecrest, CA. Kit foxes were confirmed in the study area by using infrared motion cameras (Cuddeback™ Digital, Park Falls, WI).

A live-trap grid (4 x 25, with 35 m centers; Figure 1) was established along State Route 178, approximately 15 km east of Ridgecrest, CA, in San Bernardino County (35° 38.89'N, 117° 30.30'W). The grid was located on an alluvial-colluvial slope and dominant perennials were *Ambrosia dumosa*, *Grayia spinosa*, and *Atriplex hymenelytra*. Dominant annuals include *Eriophyllum wallacei*, *Erodium cicutarium*, and *Lepidium flavum*.

[1]Murdoch, J. D. 2004. Scent marking behavior of the San Joaquin kit fox (*Vulpes macrotis mutica*). M.S. Thesis, University of Denver, Denver, Colorado, USA.

Figure 1. Small mammal trapping grid layout. Darkened circles note where kit fox scats were observed, San Bernardino County, April and May 2006.

Each rodent live-trap was placed under a cardboard A-frame shade (California Department of Fish and Game 2003[2]). The grid initially was trapped the week of 3 April 2006, and no scats were present near the trap stations. The second session was conducted the week of 15 May 2006. On 15 May, 12 kit fox scats were observed near the apex of the cardboard shades, approximately 20 cm above ground level (Figure 1). Scats were deposited sometime between 8 April and 14 May. All scats appeared similar in color and condition, and may have been deposited during one marking event.

[2]California Department of Fish and Game. 2003. California Department of Fish and Game Mohave ground squirrel survey guidelines. 5 pp.

It is not well understood why kit foxes mark novel and conspicuous objects with scats. Marking of novel objects may fall within the same behavioral category as latrine creation and use. Ralls and Smith (2004) concluded that the function of kit fox latrines is not known, but they may play a role in chemical communication. Murdoch (2004[1]) concluded that latrines probably function as a message center for canid social groups and conspecifics. O'Farrell (1987) reported that there is no evidence that kit foxes "systematically marked their territorial boundaries or sign stations with urine or feces" but scent-marking behaviors by kit foxes might play a key part in reproduction synchrony or possibly maintenance of territories. Murdoch (2004[1]) went on to state that scent marking is most likely not a function of the "bookkeeping system" in regard to marking foraging areas.

The marking of traps in Egoscue's study (1962) and the trap shades in this study are similar in that several trap locations were marked (>10%). Egoscue's (1962) small mammal trapping success dropped considerably after marking by kit fox was noted, however my trapping success did not change considerably. During the week of 3 April, 37 individual white-tailed antelope squirrels, *Ammospermophilus leucurus*, were captured, compared to 31 squirrels during the week of 15 May (H. Clark, unpub. data).

Kit foxes may mark novel objects as an exploratory action, communicating that there is something of interest in the area. In terms of small mammal traps, the marking may indicate prey in the area.

The communicative role of feces is unclear, but feces occurring on latrines and other objects may provide long-term chemical communication to conspecifics. More research is required to determine the specific functions of fecal marking of latrines and novel objects.

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FIRST RECORD OF THE OCCURRENCE OF THE CALIFORNIA GRUNION, *LEURESTHES TENUIS*, IN TOMALES BAY, CALIFORNIA: A NORTHERN EXTENSION OF THE SPECIES

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On 8 July 2005, California grunion, *Leuresthes tenuis,* were observed and collected by hand at Sand Point, Tomales Bay, Marin County, California (Latitude 38°13.9'N, Longitude 122°58.4'W). The fish were examined and their identification confirmed by the junior authors. As the previously known range of this species was from Magdalena Bay, Baja California, to San Francisco, this represents a northward expansion by a distance of approximately 40 statute miles (Miller and Lea 1972, Eschmeyer et al. 1983). Although the distance may seem insignificant, the San Francisco locale dates from the original description of the species in 1860 (Ayres 1860). We know of no records of California grunion from San Francisco or San Francisco Bay following the original description until grunion were observed there in 2001 (K. Hieb, California Department of Fish and Game, personal communication).

Approximately 30 individuals were seen at Sand Point in the 'wash zone' in small groups during a 1-hour observation period which began at 0100 hours on 8 July 2005. The fish exhibited spawning behavior typical of this species in which individuals swim onto the sandy beach with the advancing tide. Ten specimens were taken and all were deposited in the Marine Vertebrates Collection of the Scripps Institution of Oceanography (SIO 05-63). A similar run was also observed on this date at Crown State Beach, Alameda, in San Francisco Bay (K.L.M. Martin, unpublished data). Like the San Francisco Bay grunion, those observed in Tomales Bay were not seen advancing beyond the margin of the water's edge as is typically observed in southern California.

The size of the Tomales Bay grunion, 119 to 140 mm Standard Length (SL), more closely approximates the southern California coastal specimens taken in Los Angeles and Orange counties during 2005 (mean of 78 specimens was 130 mm SL) than specimens from San Francisco Bay (mean of 10 specimens was 119 mm SL) (K.L.M. Martin, unpublished data). The run was observed at a date and time predicted for grunion runs in southern California by the California Department of Fish and Game (www.dfg.ca.gov/mrd/grunschd.html).

Even though the type specimen of *Leuresthes tenuis* was taken from a fish market in San Francisco (Ayres 1860), the species is generally considered to be rare north of Point Conception. Spratt (1981) reported on spawning grunion from Monterey Bay and grunion runs have been observed almost annually at Del Monte Beach in Monterey since the mid-1990s (R.N. Lea and G. Bernardi, personal observations). Small numbers began to appear in California Department of Fish and Game midwater trawls in San Francisco Bay during 2001 (K. Hieb, personal communication; California Academy of Sciences catalog number CAS 214805). These findings were corroborated by net surveys conducted by the Port of Oakland in 2003 (A. Jahn, personal communication). Grunion runs were observed on East Bay beaches during 2005 by members of the Grunion Greeters Organization (www.grunion.org).

The senior author's interest in the northern expansion of the grunion's range was piqued by local citizens' reports of a grunion run in Tomales Bay during June 2005. Initially, these reports were discounted as sightings of smelts (Osmeridae) or New World silversides (Atherinopsidae; topsmelt and jacksmelt) which are common in Tomales Bay. However, an interview with a local resident who described typical California grunion spawning behavior during the time of a predicted grunion run lent credence to these reports.

While it may be possible that grunion occurred in previous years in Tomales Bay, it is unlikely. A comprehensive literature and museum search by the National Park Service's Inventory and Monitoring Program to list species occurring at the Point Reyes National Seashore (including Tomales Bay) failed to locate any references to *Leuresthes tenuis*. Sand Point, Tomales Bay, where the grunion were seen, is part of the Lawson's Landing Resort, a trailer park and campground that has been in operation since 1957. If there were grunion runs in other years, it is likely that the fish would have been observed by campers at the resort as they were in 2005. An elder of the family that owns the resort, who is in his 80s, reported that 2005 was the only year he has ever seen grunion in Tomales Bay.

While beach spawning is not common, it has evolved many times in fishes all over the world (Martin et al. 2004), and is probably driven by access to particular oviposition sites and substrates combined with plastic reproductive behavior (Martin and Swiderski 2001). The grunion possibly took advantage of the unusually light winds and favorable ocean conditions of spring 2005 to transit the inhospitable outer coast of Marin County and take up residence in the relatively benign and warmer waters of Tomales Bay.

The northern expansion of the California grunion's range is just one of a number of observations coincident with unusual ocean conditions which were documented during spring-summer 2005 along the West Coast of North America by a number of

investigations. While oceanographers have characterized the period since 1998 as a 'cold water' period (Peterson and Schwing 2004), in 2005 the California Current exhibited anomalously warm sea surface temperatures. Delayed onset of upwelling, which normally occurs in the spring but didn't commence until June-July in 2005, was generally held to be responsible for the warm-water condition. During the spring, the weak upwelling led to lower than normal primary production (Schwing et al. 2006). Coincident with low production was an unprecedented mass abandonment of Cassin's auklet nests on the Farallon Islands (Sydeman et al. 2006) and record low catches of young-of-year rockfishes off central California by the National Marine Fisheries Service juvenile rockfish recruitment survey (Brodeur et al. 2006). The San Francisco Bay herring fishery caught very few fish in 2005 relative to other years. (D. Watters, California Department of Fish and Game, personal communication).

Warm water anomalies are known to be accompanied by latitudinal range shifts of southern fishes into northern waters (Lea and Rosenblatt 2000). While the warm-water episode of 2005 was not characterized as an El Niño, it is possible that the northward movement of *Leuresthes tenuis* is indicative of a poorly known element of the California Current system which we are only beginning to understand.

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Postscript: Observers returned to Tomales Bay during the summer of 2006 and witnessed hundreds of fish spawning at Sand Point on 28 June and thousands of fish on the beach 27 July.

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